

Last Update: February 4, 2025



WebChatter

Version 1.0.1

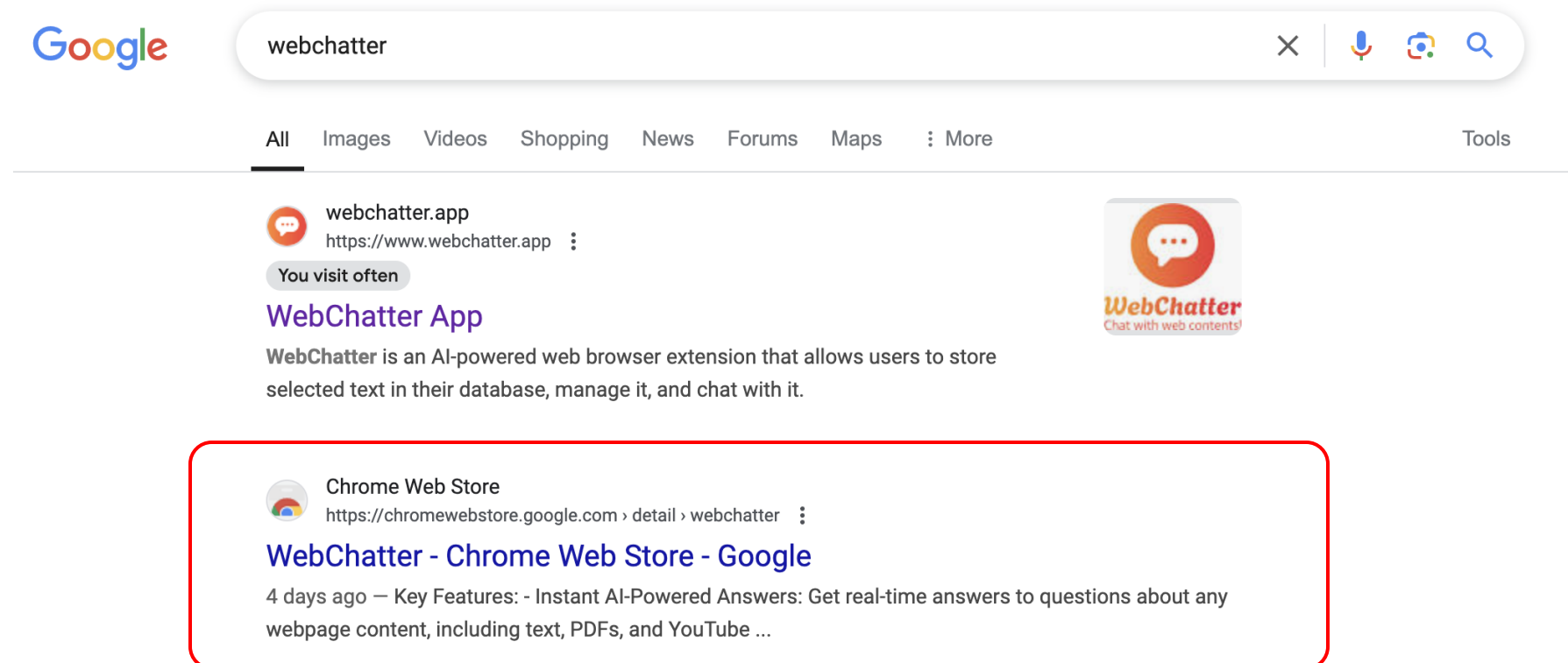
How to use WebChatter App



Available in the
Chrome Web Store

Database: <https://www.webchatter.app>

Google “WebChatter”




The image shows a Google search interface. The search bar contains the text "webchatter". Below the search bar, there are navigation tabs for "All", "Images", "Videos", "Shopping", "News", "Forums", "Maps", and "More". The "All" tab is selected. The search results are displayed below the tabs. The first result is for "webchatter.app" with the URL "https://www.webchatter.app". It has a "You visit often" badge and a purple title "WebChatter App". The description states: "WebChatter is an AI-powered web browser extension that allows users to store selected text in their database, manage it, and chat with it." To the right of the text is a logo for "WebChatter" with the tagline "Chat with web contents!". The second result is from the "Chrome Web Store" with the URL "https://chromewebstore.google.com > detail > webchatter". It has a blue title "WebChatter - Chrome Web Store - Google" and a description: "4 days ago – Key Features: - Instant AI-Powered Answers: Get real-time answers to questions about any webpage content, including text, PDFs, and YouTube ...". This second result is enclosed in a red rounded rectangle.

Google

webchatter


All Images Videos Shopping News Forums Maps : More Tools


 webchatter.app
https://www.webchatter.app

You visit often

WebChatter App

WebChatter is an AI-powered web browser extension that allows users to store selected text in their database, manage it, and chat with it.

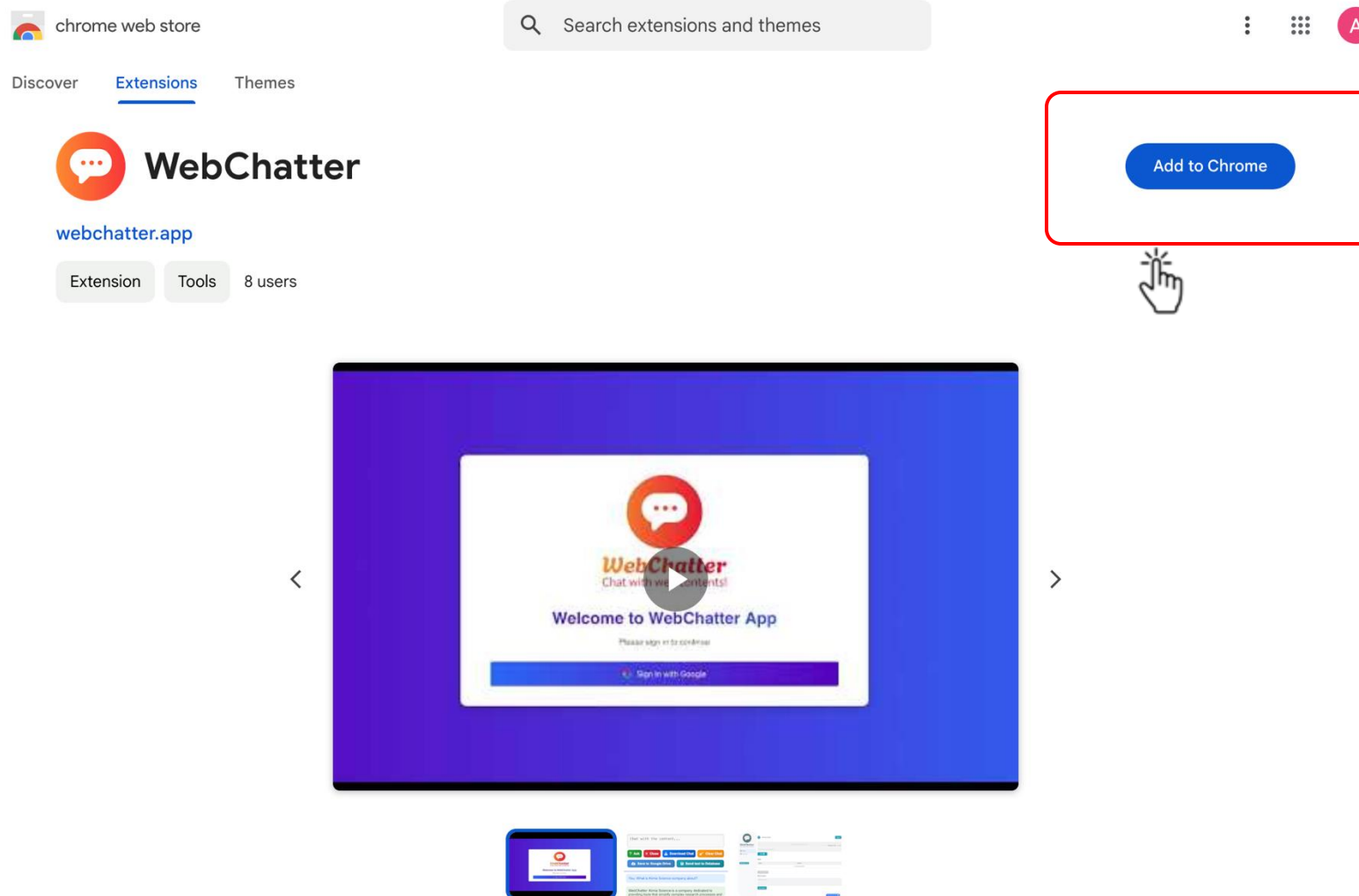


 Chrome Web Store
https://chromewebstore.google.com > detail > webchatter

WebChatter - Chrome Web Store - Google

4 days ago – Key Features: - Instant AI-Powered Answers: Get real-time answers to questions about any webpage content, including text, PDFs, and YouTube ...


Add WebChatter to Chrome



chrome web store

Search extensions and themes

Discover Extensions Themes

 **WebChatter**

webchatter.app

Extension Tools 8 users

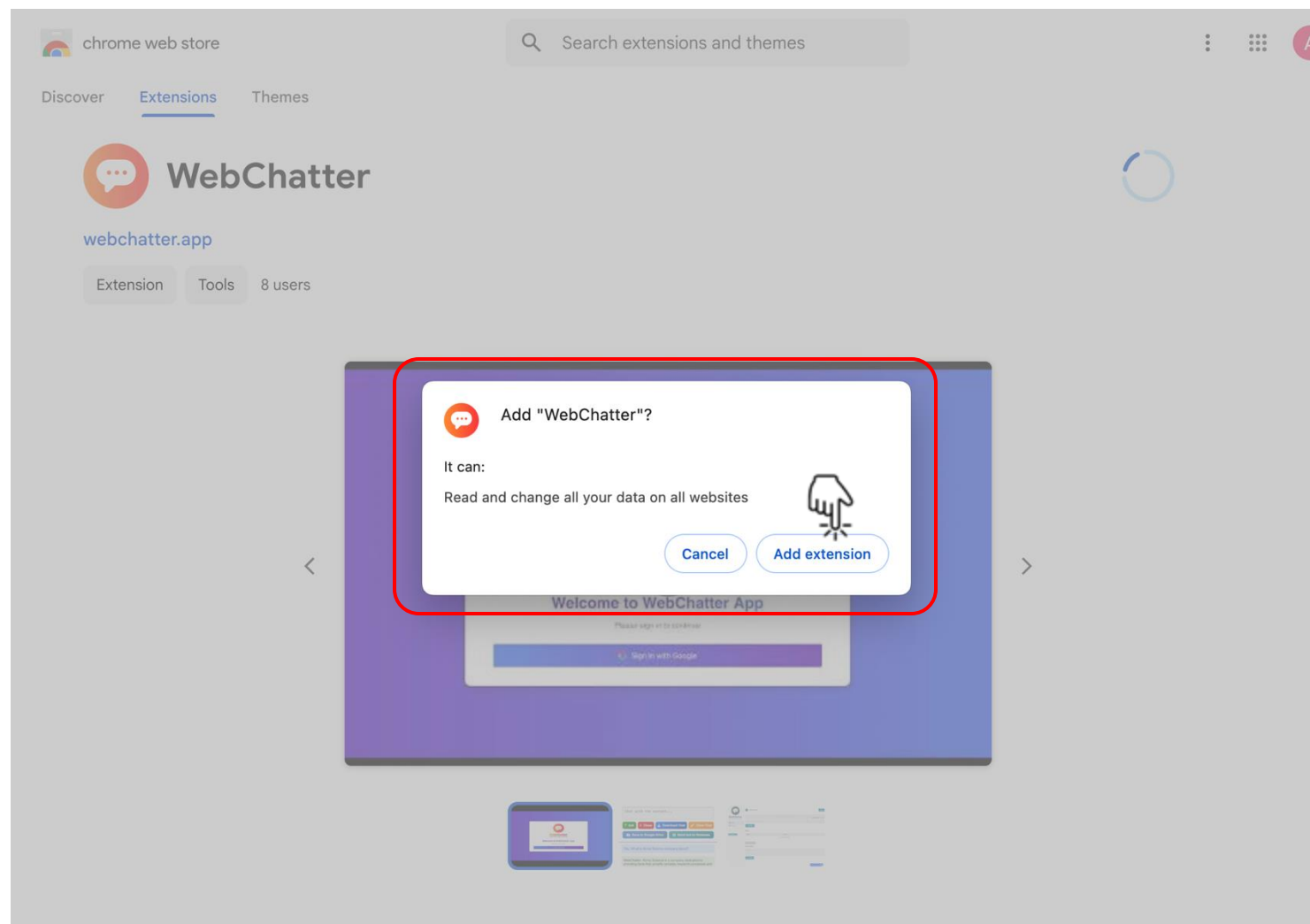
Add to Chrome

Welcome to WebChatter App

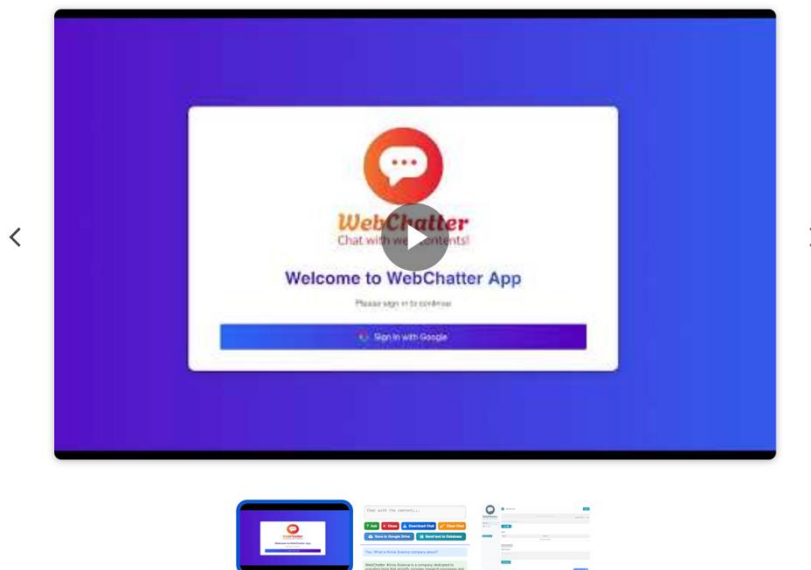
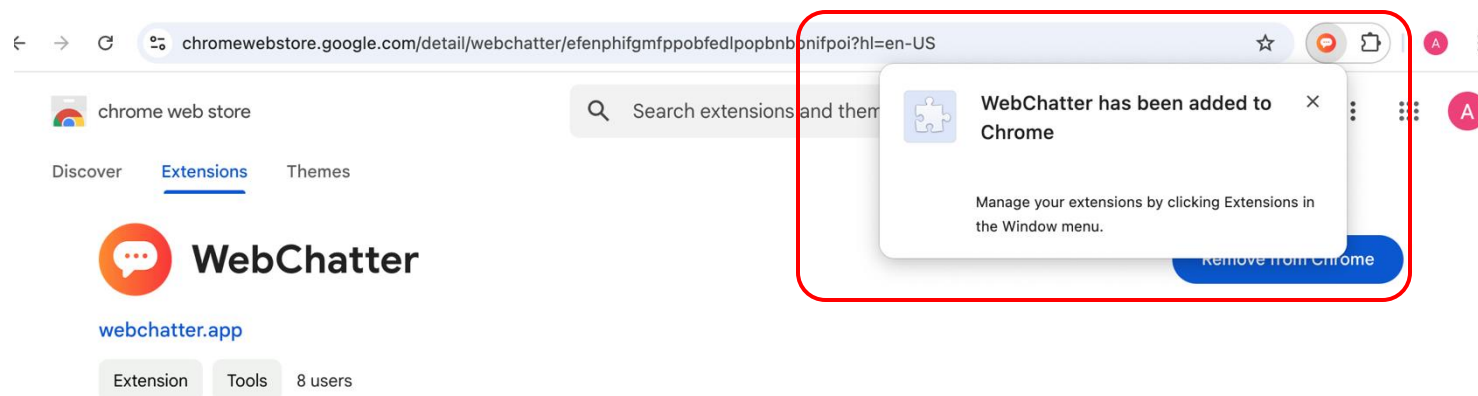
Please sign in to continue

Sign in with Google

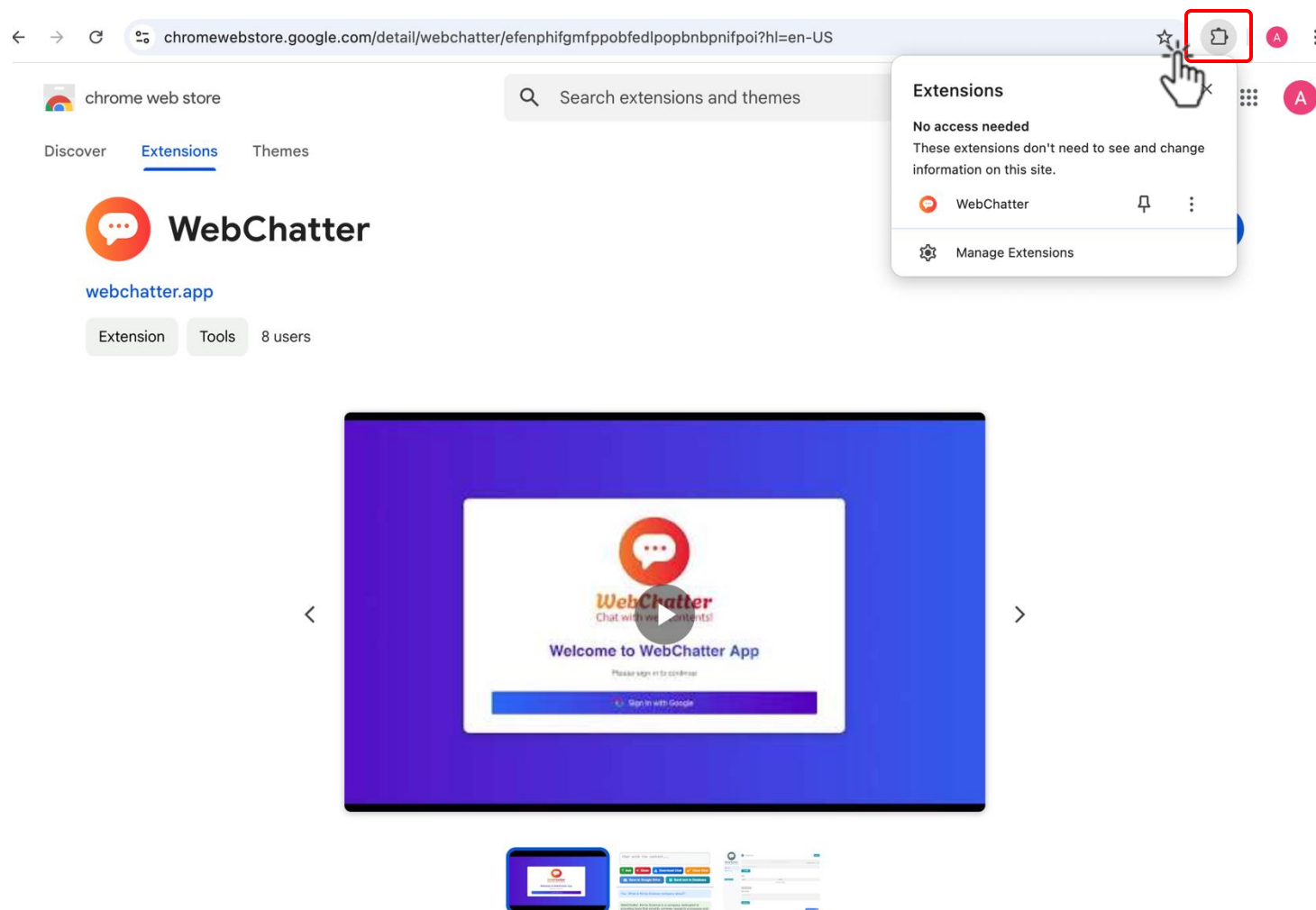
Add the extension



Added ...



From Extensions icon




The image shows a Chrome browser window displaying the Chrome Web Store page for the 'WebChatter' extension. The address bar shows the URL: chromewebstore.google.com/detail/webchatter/efenphifgmfpobfedlpopbnpnifpoi?hl=en-US. The page title is 'chrome web store' and the search bar contains 'Search extensions and themes'. The 'WebChatter' extension is listed with a red speech bubble icon, the URL 'webchatter.app', and the category 'Extension' with '8 users'. A red box highlights the 'Extensions' icon in the top right corner of the browser. A hand cursor is pointing at the icon, and a dropdown menu is open, showing the 'Extensions' title, a 'No access needed' warning, the 'WebChatter' extension with a pin icon, and a 'Manage Extensions' option.

chrome web store

Search extensions and themes

Discover Extensions Themes




 **WebChatter**


webchatter.app

Extension Tools 8 users

Extensions

No access needed
These extensions don't need to see and change information on this site.

 WebChatter  

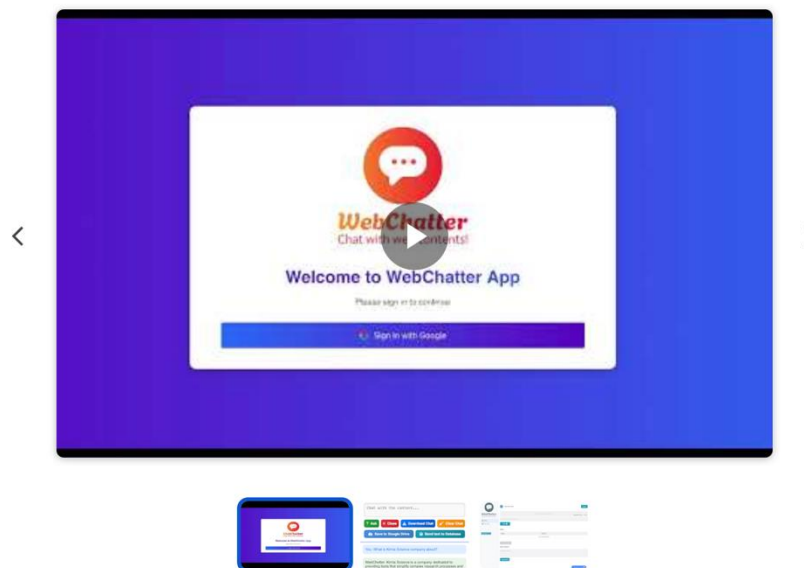
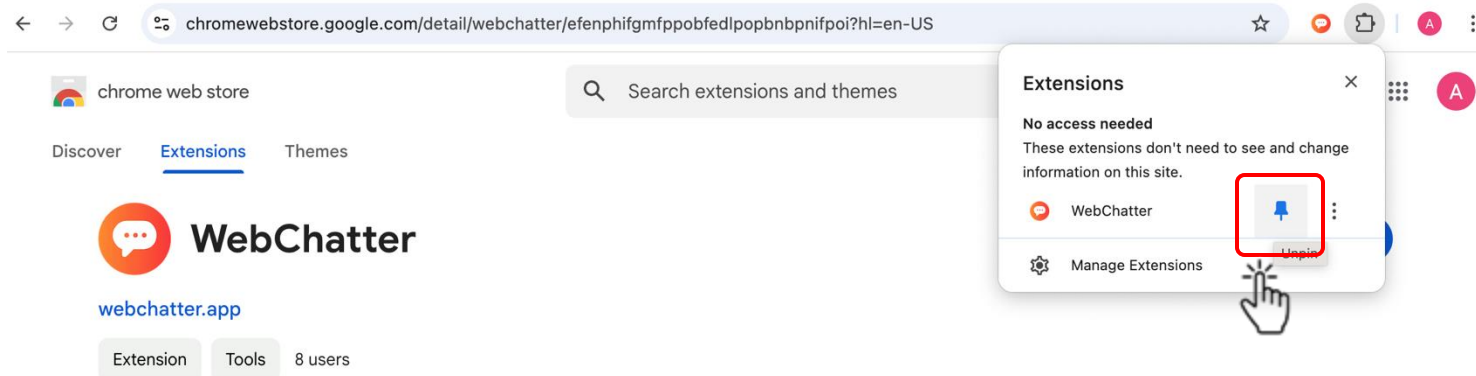
 Manage Extensions

Welcome to WebChatter App

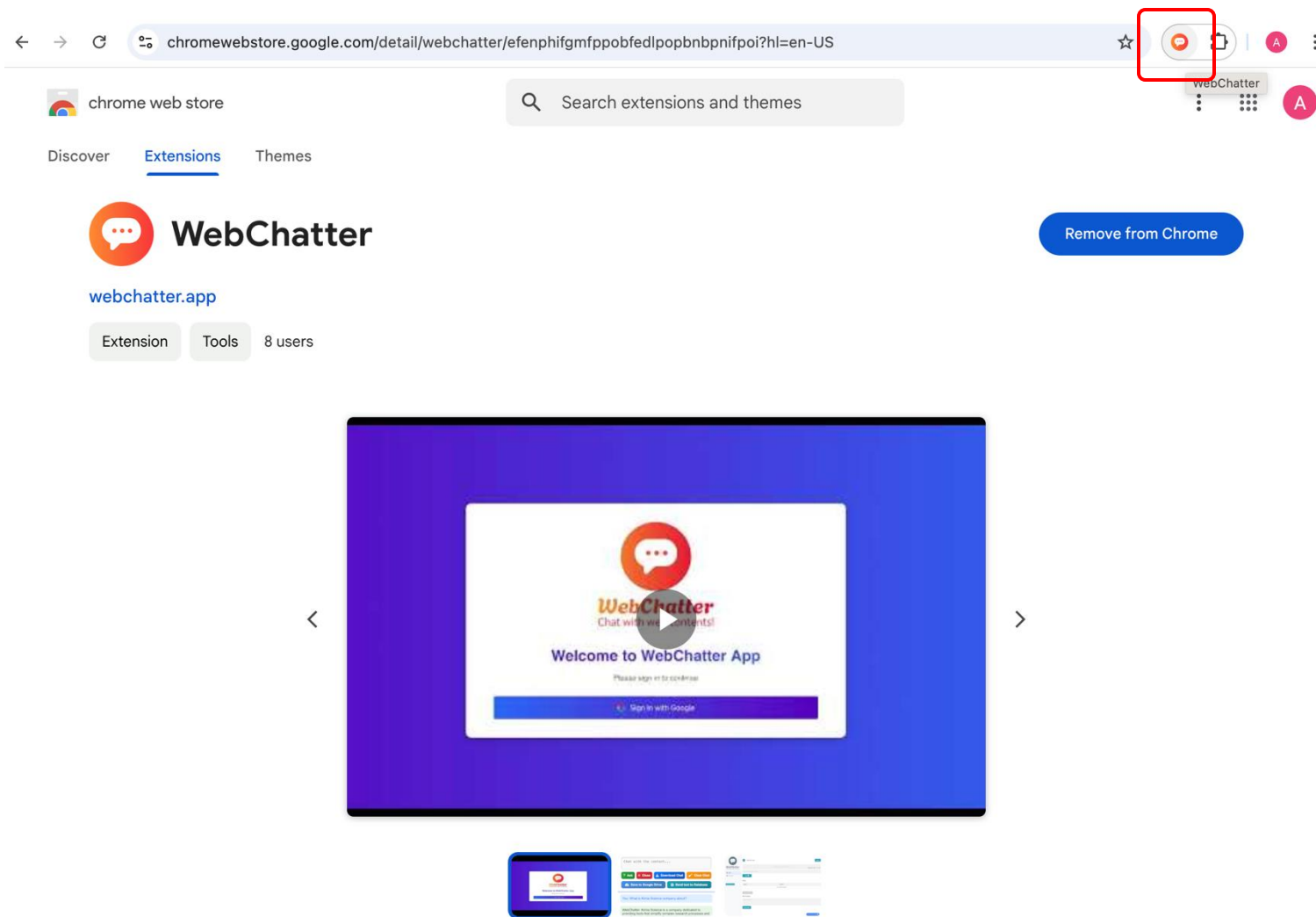
Please sign in to continue

[Sign in with Google](#)

Pin the extension

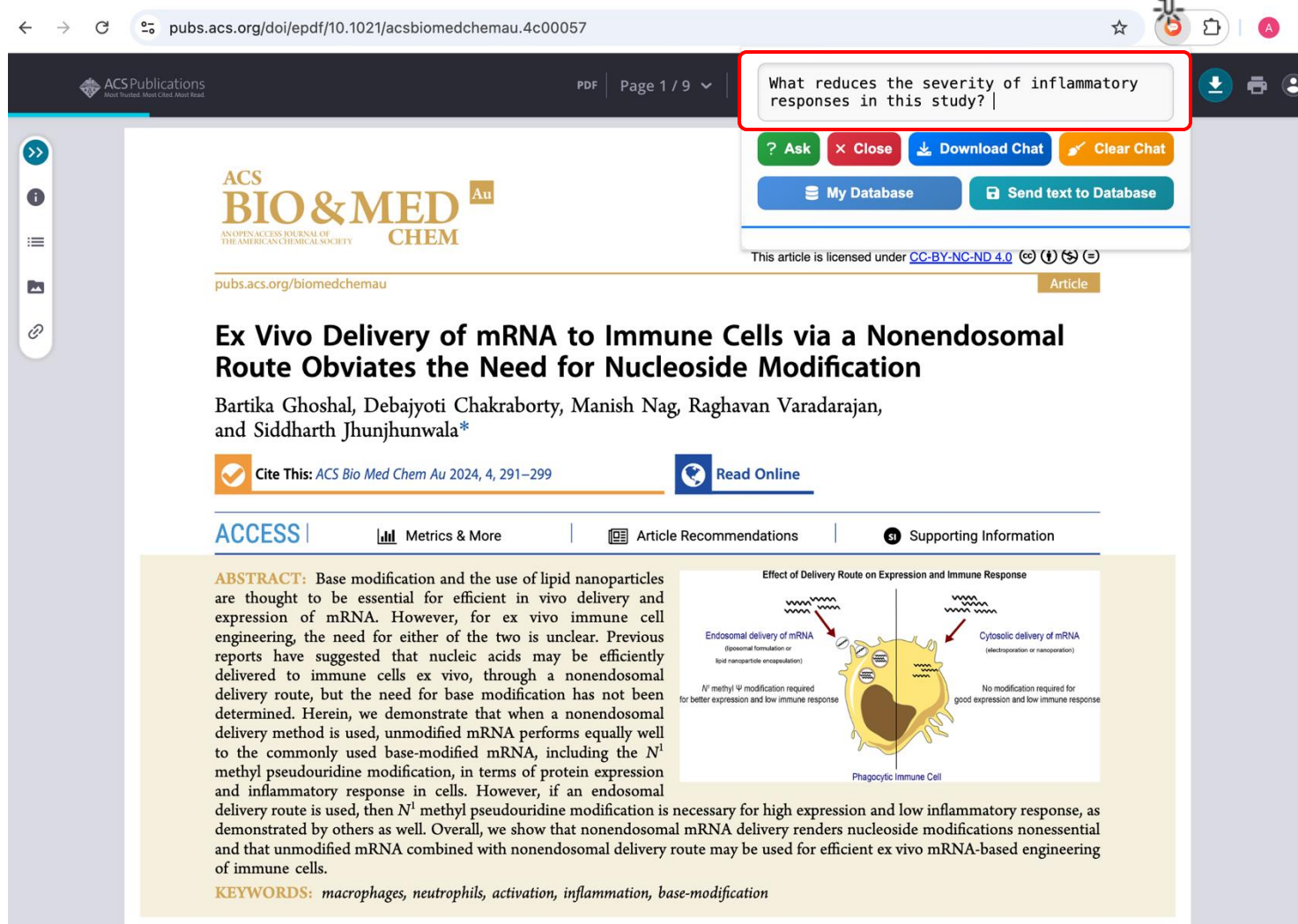


Pinned ...



The image shows a screenshot of the Chrome Web Store page for the 'WebChatter' extension. The browser's address bar shows the URL: chromewebstore.google.com/detail/webchatter/efenphifgmfpobfedlpopbnpnifpoi?hl=en-US. The extension is listed as 'WebChatter' with the URL 'webchatter.app'. It is categorized as an 'Extension' and 'Tools' and has '8 users'. A 'Remove from Chrome' button is visible. Below the extension details is a large preview image of the WebChatter app interface, which features a blue and purple gradient background and a central white box with the WebChatter logo and the text 'Welcome to WebChatter App'. A 'Sign in with Google' button is also present in the preview. The browser's toolbar shows the extension icon pinned to the right side of the address bar, highlighted with a red box.

Type in any questions you have about the content, click **Ask**, or press Enter / return



The screenshot shows a web browser displaying an ACS Publications article. A chat window is open at the top right, containing the question: "What reduces the severity of inflammatory responses in this study?". Below the question are buttons for "Ask", "Close", "Download Chat", and "Clear Chat". There are also buttons for "My Database" and "Send text to Database".

The article title is "Ex Vivo Delivery of mRNA to Immune Cells via a Nonendosomal Route Obviates the Need for Nucleoside Modification" by Bartika Ghoshal, Debajyoti Chakraborty, Manish Nag, Raghavan Varadarajan, and Siddharth Jhunjhunwala*. The article is licensed under CC-BY-NC-ND 4.0.

The abstract states: "Base modification and the use of lipid nanoparticles are thought to be essential for efficient in vivo delivery and expression of mRNA. However, for ex vivo immune cell engineering, the need for either of the two is unclear. Previous reports have suggested that nucleic acids may be efficiently delivered to immune cells ex vivo, through a nonendosomal delivery route, but the need for base modification has not been determined. Herein, we demonstrate that when a nonendosomal delivery method is used, unmodified mRNA performs equally well to the commonly used base-modified mRNA, including the N¹ methyl pseudouridine modification, in terms of protein expression and inflammatory response in cells. However, if an endosomal delivery route is used, then N¹ methyl pseudouridine modification is necessary for high expression and low inflammatory response, as demonstrated by others as well. Overall, we show that nonendosomal mRNA delivery renders nucleoside modifications nonessential and that unmodified mRNA combined with nonendosomal delivery route may be used for efficient ex vivo mRNA-based engineering of immune cells."

KEYWORDS: macrophages, neutrophils, activation, inflammation, base-modification

The diagram titled "Effect of Delivery Route on Expression and Immune Response" shows a phagocytic immune cell. On the left, "Endosomal delivery of mRNA (liposomal formulation or lipid nanoparticle encapsulation)" is shown with a note: "N¹ methyl Ψ modification required for better expression and low immune response". On the right, "Cytosolic delivery of mRNA (selectoporation or nanoporation)" is shown with a note: "No modification required for good expression and low immune response".

The question and its answer pups up

pubs.acs.org/doi/epdf/10.1021/acsbioimedchemau.4c00057

ACS Publications
PDF | Page 1 / 9

Chat with the content...

Ask Close Download Chat Clear Chat

My Database Send text to Database

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THE AMERICAN CHEMICAL SOCIETY
CHEM

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Ex Vivo Delivery of mRNA to Immune Cells via a Nonendosomal Route Obviates the Need for Nucleoside Modifications

Bartika Ghoshal, Debajyoti Chakraborty, Manish Nag, Raghavendra Prasad, and Siddharth Jhunjhunwala*

Cite This: *ACS Bio Med Chem Au* 2024, 4, 291–299

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ABSTRACT: Base modification and the use of lipid nanoparticles are thought to be essential for efficient in vivo delivery and expression of mRNA. However, for ex vivo immune cell engineering, the need for either of the two is unclear. Previous reports have suggested that nucleic acids may be efficiently delivered to immune cells ex vivo, through a nonendosomal delivery route, but the need for base modification has not been determined. Herein, we demonstrate that when a nonendosomal delivery method is used, unmodified mRNA performs equally well to the commonly used base-modified mRNA, including the N^1 methyl pseudouridine modification, in terms of protein expression and inflammatory response in cells. However, if an endosomal delivery route is used, then N^1 methyl pseudouridine modification is necessary for high expression and low inflammatory response, as demonstrated by others as well. Overall, we show that nonendosomal mRNA delivery renders nucleoside modifications nonessential and that unmodified mRNA combined with nonendosomal delivery route may be used for efficient ex vivo mRNA-based engineering of immune cells.

KEYWORDS: macrophages, neutrophils, activation, inflammation, base-modification

Effect of Delivery Route on Expression and Immune Response



Endosomal delivery of mRNA (lipid nanoparticle encapsulation)

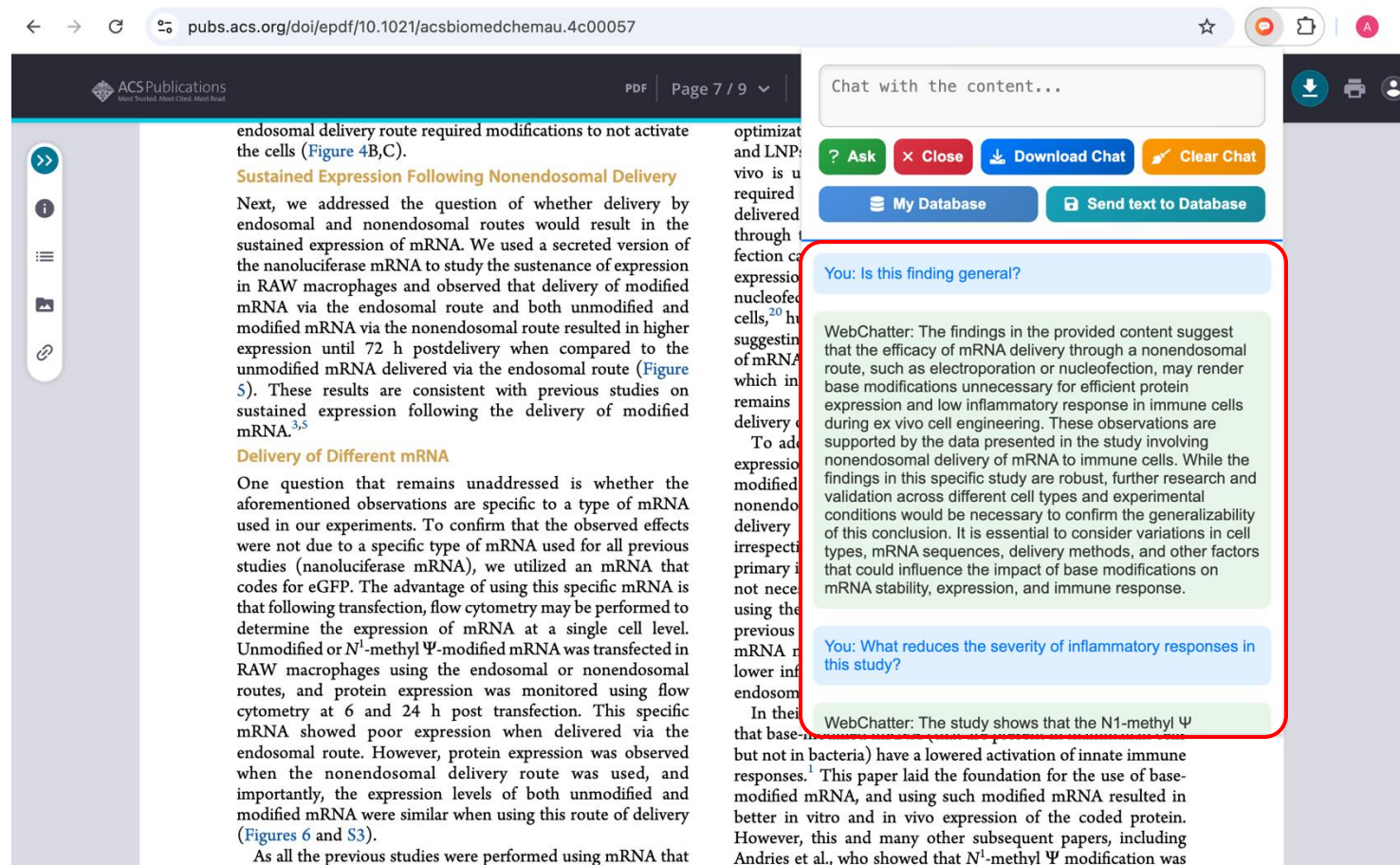
Cytosolic delivery of mRNA (electroporation or nanoporation)

N^1 methyl Ψ modification required for better expression and low immune response

No modification required for good expression and low immune response

Phagocytic Immune Cell

Continue if you have other questions



pubs.acs.org/doi/epdf/10.1021/acsbiochemau.4c00057

ACS Publications
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PDF | Page 7 / 9

Chat with the content...

Ask
Close
Download Chat
Clear Chat

My Database
Send text to Database

You: Is this finding general?

WebChatter: The findings in the provided content suggest that the efficacy of mRNA delivery through a nonendosomal route, such as electroporation or nucleofection, may render base modifications unnecessary for efficient protein expression and low inflammatory response in immune cells during ex vivo cell engineering. These observations are supported by the data presented in the study involving nonendosomal delivery of mRNA to immune cells. While the findings in this specific study are robust, further research and validation across different cell types and experimental conditions would be necessary to confirm the generalizability of this conclusion. It is essential to consider variations in cell types, mRNA sequences, delivery methods, and other factors that could influence the impact of base modifications on mRNA stability, expression, and immune response.

You: What reduces the severity of inflammatory responses in this study?

WebChatter: The study shows that the N1-methyl Ψ (but not in bacteria) have a lowered activation of innate immune responses.¹ This paper laid the foundation for the use of base-modified mRNA, and using such modified mRNA resulted in better in vitro and in vivo expression of the coded protein. However, this and many other subsequent papers, including Andries et al., who showed that N¹-methyl Ψ modification was

endosomal delivery route required modifications to not activate the cells (Figure 4B,C).

Sustained Expression Following Nonendosomal Delivery

Next, we addressed the question of whether delivery by endosomal and nonendosomal routes would result in the sustained expression of mRNA. We used a secreted version of the nanoluciferase mRNA to study the sustenance of expression in RAW macrophages and observed that delivery of modified mRNA via the endosomal route and both unmodified and modified mRNA via the nonendosomal route resulted in higher expression until 72 h postdelivery when compared to the unmodified mRNA delivered via the endosomal route (Figure 5). These results are consistent with previous studies on sustained expression following the delivery of modified mRNA.^{3,5}

Delivery of Different mRNA

One question that remains unaddressed is whether the aforementioned observations are specific to a type of mRNA used in our experiments. To confirm that the observed effects were not due to a specific type of mRNA used for all previous studies (nanoluciferase mRNA), we utilized an mRNA that codes for eGFP. The advantage of using this specific mRNA is that following transfection, flow cytometry may be performed to determine the expression of mRNA at a single cell level. Unmodified or N¹-methyl Ψ -modified mRNA was transfected in RAW macrophages using the endosomal or nonendosomal routes, and protein expression was monitored using flow cytometry at 6 and 24 h post transfection. This specific mRNA showed poor expression when delivered via the endosomal route. However, protein expression was observed when the nonendosomal delivery route was used, and importantly, the expression levels of both unmodified and modified mRNA were similar when using this route of delivery (Figures 6 and S3).

As all the previous studies were performed using mRNA that

optimizat and LNP: vivo is u required delivered through t fection ca expression nucleofec cells,²⁰ in suggesti of mRNA which in remains delivery c To ad expression modified nonendo delivery irrespect primary i not nece using the previous mRNA r lower inf endosom In thei that base-m but not in bacteria) have a lowered activation of innate immune responses.¹ This paper laid the foundation for the use of base-modified mRNA, and using such modified mRNA resulted in better in vitro and in vivo expression of the coded protein. However, this and many other subsequent papers, including Andries et al., who showed that N¹-methyl Ψ modification was

Send selected text to database

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PDF Page 2 / 9

analogue (Sapala Organics Private Limited, Hyderabad, India) and 7.5 mM of GTP.

The reaction was set up at 37 °C for 2.5 h, after which DNase treatment was performed to remove any linear DNA contaminants. This was followed by lithium chloride precipitation to precipitate the RNA, which was then reconstituted in nuclease-free water and stored at -80 °C for transfections. In the case of Cypridina luciferase mRNA, the modified mRNA was purchased from RNAVaxBio (Himachal Pradesh, India).

mRNA Transfection on Mammalian and Primary Cells

Lipofectamine (Endosomal) Method. Cells were transfected using Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific), following the manufacturer's instructions. RAW or dHL60 cells were transfected with 800 ng of nanoluciferase mRNA (the nonsecretory expressing form) in a 24-well plate with P3000 and reduced serum media, OptiMeM (Gibco, Thermo Fisher Scientific). The nanoluciferase transfections were done in duplicates, and the average luminescence reading was tabulated for each time point. For Cypridina luciferase and nanoluciferase (secretory expressing form) transfection, 1.5 μg was transfected in a 12-well plate with P3000, OptiMeM and luminescence was recorded once for each independent set. In the case

mercaptoethanol and 40 ng/mL of GM-CSF (Granulocyte-macrophage colony stimulating factor-R&D Systems). On day 2, the media was replaced with fresh media and 20 ng/mL of GM-CSF. On day 4, only half the media was replaced with fresh media and 20 ng/mL GM-CSF. On day 6, the cells were scraped and seeded in a 12-well plate for transfection on the following day, with growth media devoid of GM-CSF.

Nanoluciferase and Cypridina Luciferase Assay

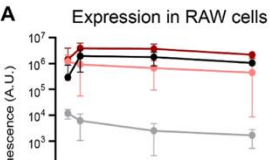
For nanoluciferase assay, the cells were harvested and resuspended in 50 μL of 1× PBS and transferred to a 96-well black plate (SPL Life Sciences, South Korea). For the assay, a Nano-Glo Luciferase Assay System (Promega, USA) was used. To make the nanoluciferase assay reagent, one volume of assay substrate was mixed with 50 volumes of assay buffer. Then, an equal volume of nanoluciferase assay reagent was added to the cell suspension, and the luminescence was read in a microplate reader (Tecan) immediately. To detect the secreted form of the mRNA, equal volumes of cell supernatant and assay reagent were added for luminescence detection.

For Cypridina luciferase, a Pierce Cypridina Luciferase Flash Assay Kit (Thermo Fisher Scientific) was used for luciferase detection. The supernatants of transfected cells were collected, and 20 μL was

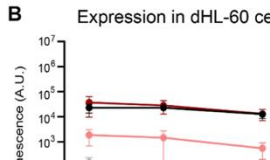
292 <https://doi.org/10.1021/acsbiochemau.4c00057>
ACS Bio Med Chem Au 2024, 4, 291–299

ACS Bio & Med Chem Au pubs.acs.org/biochemau Article


A Expression in RAW cells



B Expression in dHL-60 cells



Open extension interface and send



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PDF | Page 2 / 9

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The reaction was set up at 37 °C for 2.5 h, after which DNase treatment was performed to remove any linear DNA contaminants. This was followed by lithium chloride precipitation to precipitate the RNA, which was then reconstituted in nuclease-free water and stored at -80 °C for transfections. In the case of Cypridina luciferase mRNA, the modified mRNA was purchased from RNAVaxBio (Himachal Pradesh, India).

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Chat with the content...

Ask Close Download Chat Clear Chat

My Database Send text to Database

You: What reduces the severity of inflammatory responses in this study?

WebChatter: The study shows that the N1-methyl Ψ modification of mRNA reduced the severity of inflammatory responses in the immune cells studied.

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A Expression in RAW cells

Expression (A.U.)

B Expression in dHL-60 cells

Expression (A.U.)

Sent ...

pubs.acs.org/doi/epdf/10.1021/acsbiomedchemau.4c00057

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PDF Page 2 / 9

Chat with the content...

Download Chat Clear Chat

Send text to Database

The extension WebChatter says
Content saved successfully
OK

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292

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A Expression in RAW cells

B Expression in dHL-60 cells

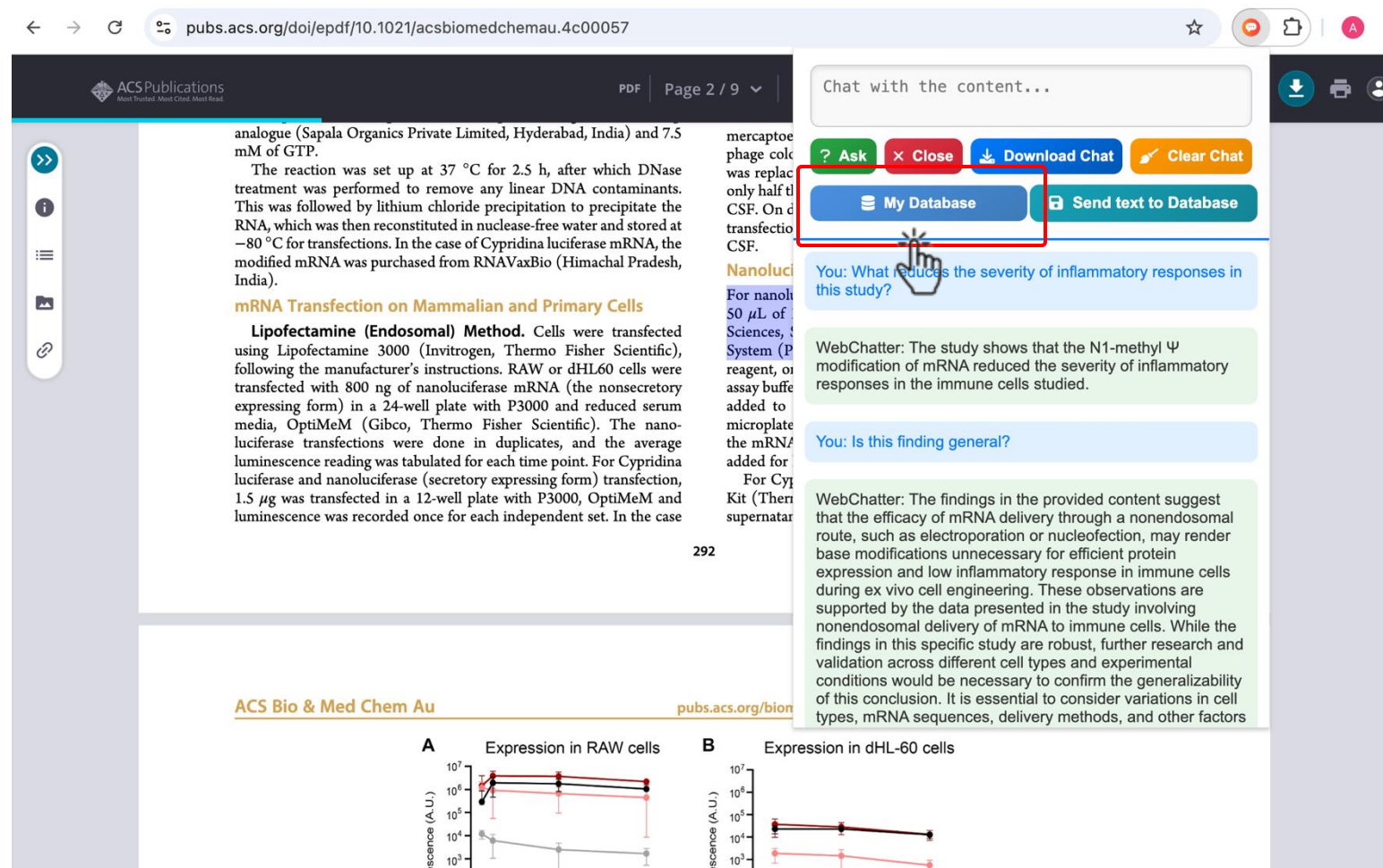
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Database web interface



The screenshot shows a web browser displaying an ACS Publications article. The URL is pubs.acs.org/doi/epdf/10.1021/acsbiochemau.4c00057. The page is titled "Page 2 / 9".

The article text includes:

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mercaptopurine (Sigma, St. Louis, MO) was added to the culture medium. The phage cocktail was replaced every 2 days. The culture medium was replaced only half the volume every 2 days. On day 7, the culture medium was replaced with fresh transfection medium.

Nanoluciferase
For nanoluciferase, 50 μL of the nanoluciferase mRNA (the nonsecretory expressing form) was added to the assay buffer. The assay buffer was added to the microplate. The mRNA was added for 24 h. For Cypridina luciferase and nanoluciferase (secretory expressing form) transfection, 1.5 μg was transfected in a 12-well plate with P3000, OptiMeM and luminescence was recorded once for each independent set. In the case

292

ACS Bio & Med Chem Au pubs.acs.org/biochemau

The chat interface overlay includes a search bar "Chat with the content...", buttons for "Ask", "Close", "Download Chat", "Clear Chat", "My Database", and "Send text to Database". A hand cursor is pointing at the "My Database" button.

Chat history:

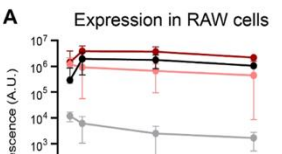
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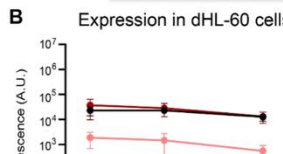
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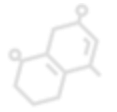
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A Expression in RAW cells

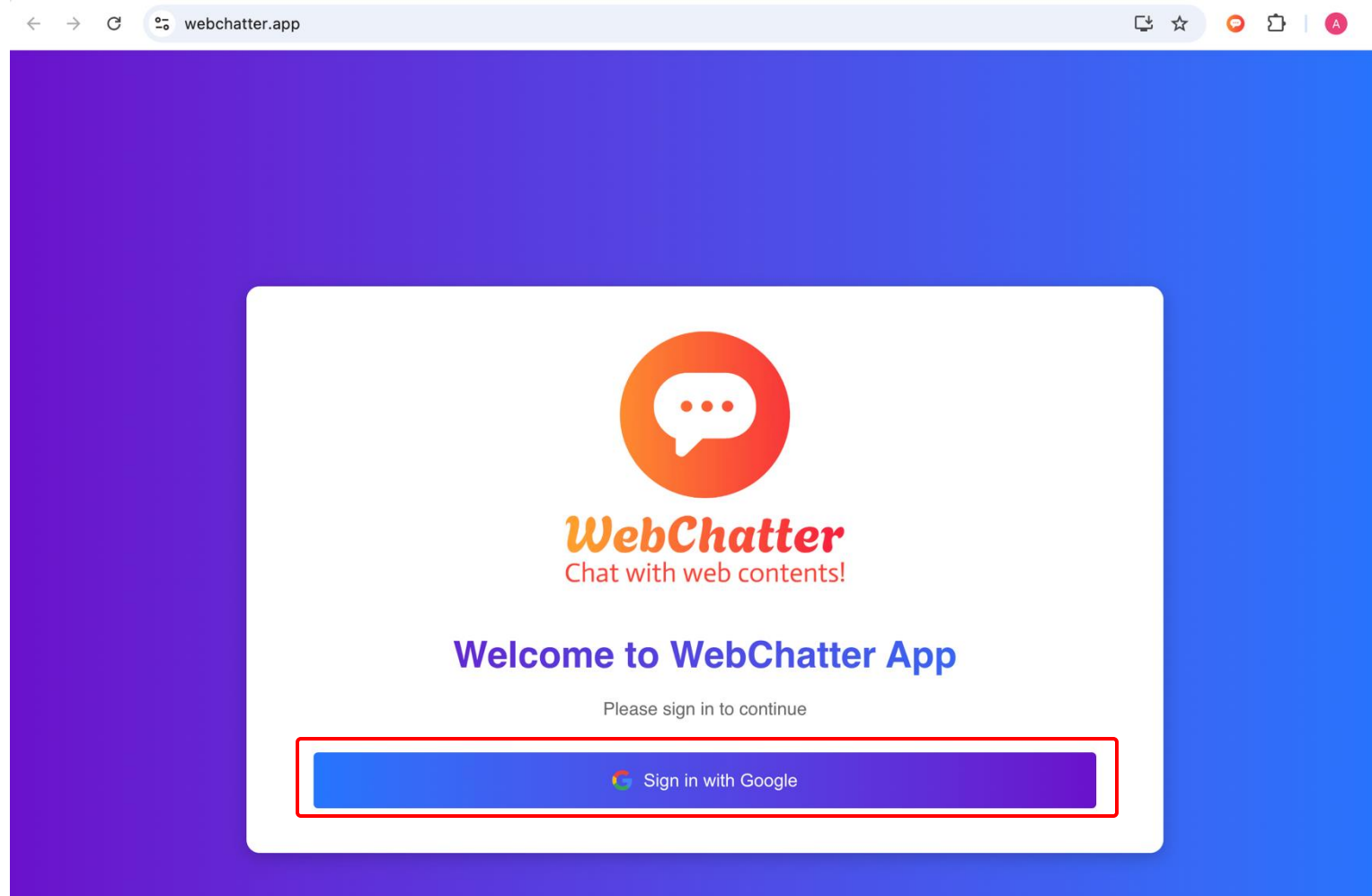


B Expression in dHL-60 cells

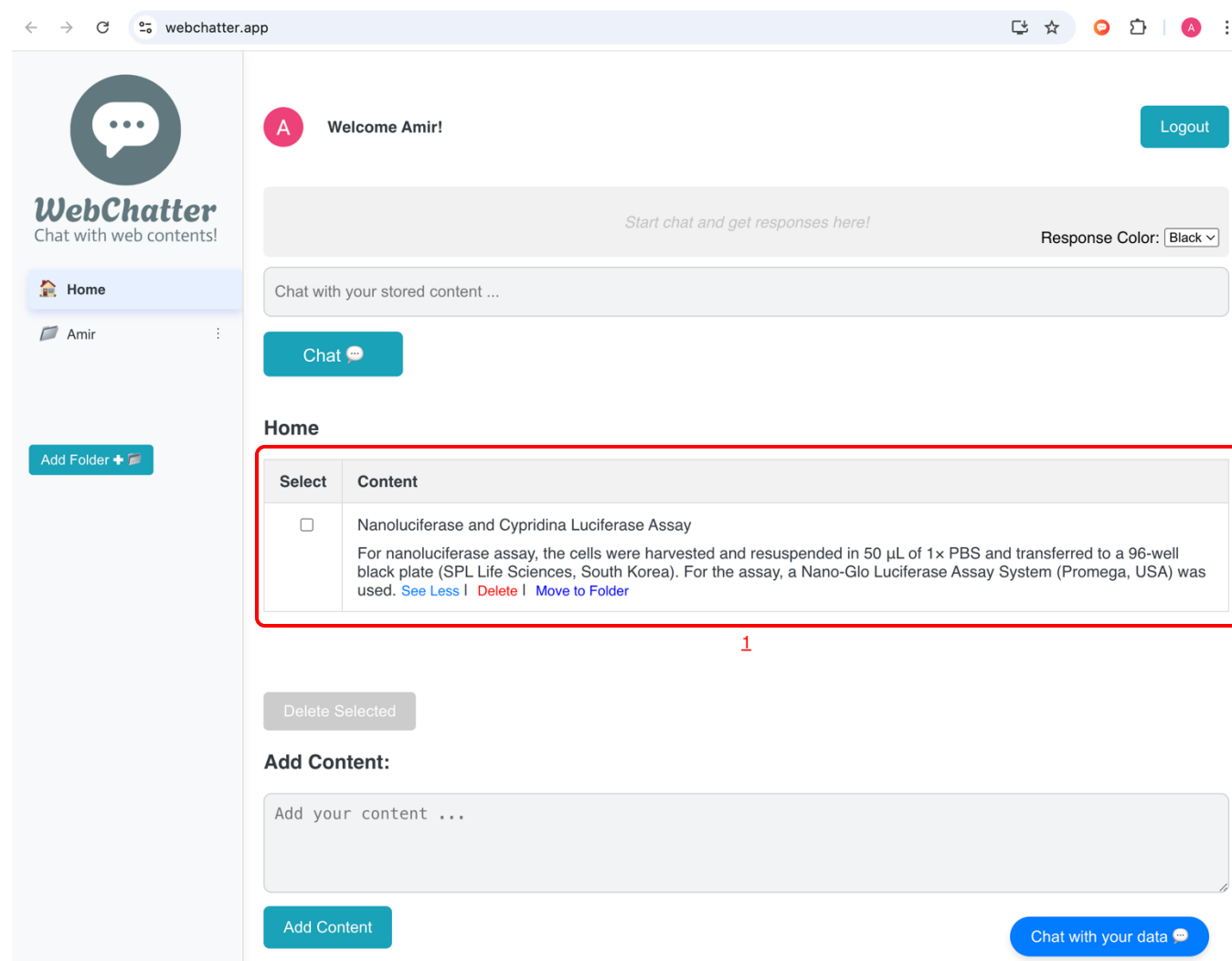




Login “webchatter.app” using same account



Selected text goes to “Home” directory



webchatter.app

Welcome Amir! Logout

Start chat and get responses here! Response Color: Black

Chat with your stored content ...

Chat

Home

Select	Content
<input checked="" type="checkbox"/>	<p>Nanoluciferase and Cypridina Luciferase Assay</p> <p>For nanoluciferase assay, the cells were harvested and resuspended in 50 μL of 1x PBS and transferred to a 96-well black plate (SPL Life Sciences, South Korea). For the assay, a Nano-Glo Luciferase Assay System (Promega, USA) was used. See Less Delete Move to Folder</p>

1

Delete Selected

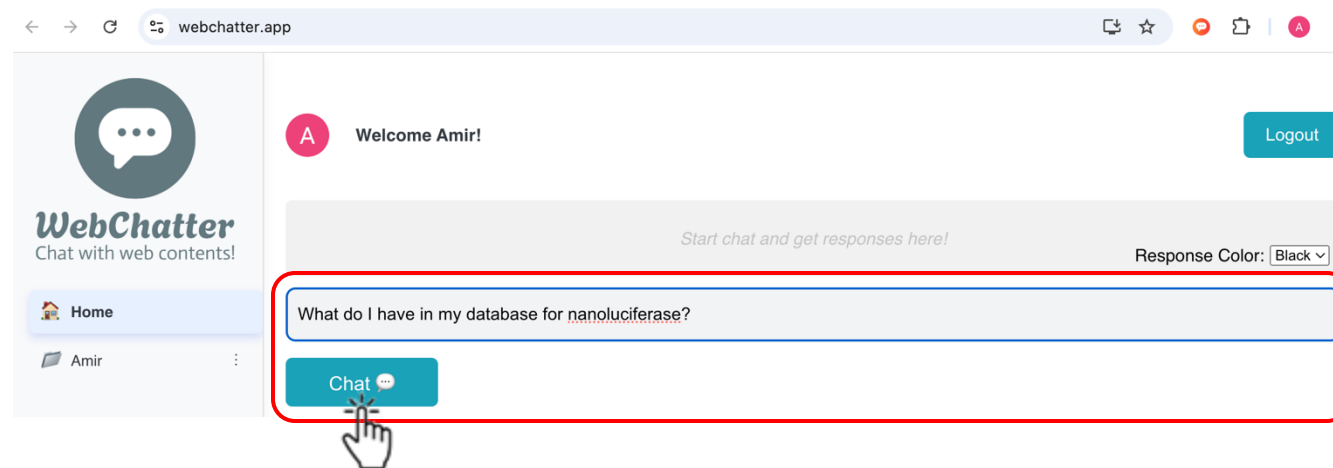
Add Content:

Add your content ...

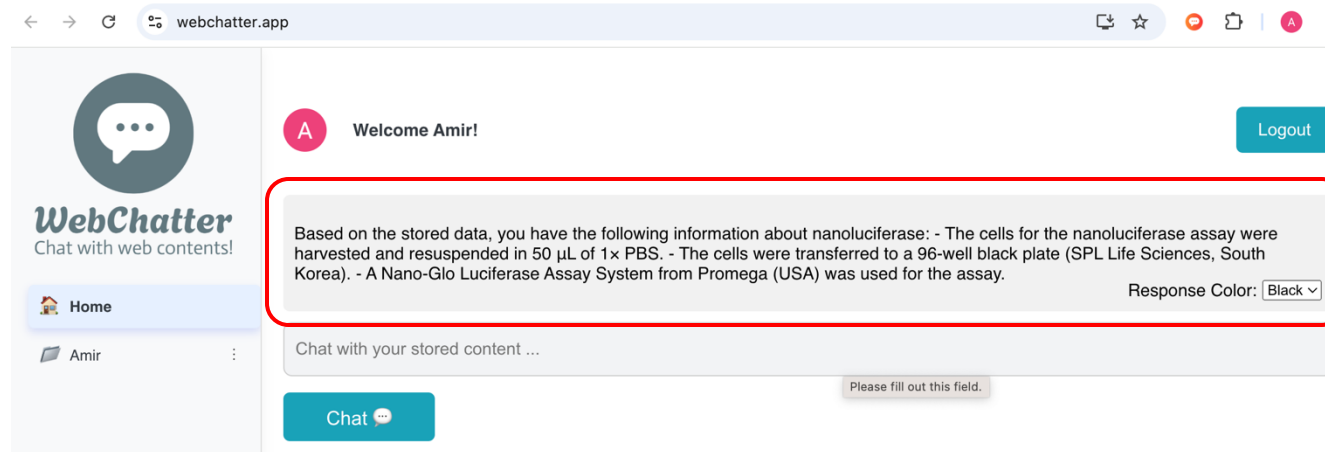
Add Content

Chat with your data

Chat with stored data: (1) docked chat



The answer pups up



The screenshot shows a web browser window with the address bar displaying "webchatter.app". The application interface includes a sidebar on the left with a "WebChatter" logo and a "Home" button. The main chat area shows a welcome message "Welcome Amir!" and a "Logout" button. A red box highlights a chat response containing text about nanoluciferase assay data and a "Response Color" dropdown menu set to "Black". Below the chat area is a text input field with a "Please fill out this field." error message and a "Chat" button.

webchatter.app

WebChatter
Chat with web contents!

Home

Amir

Welcome Amir!

Logout

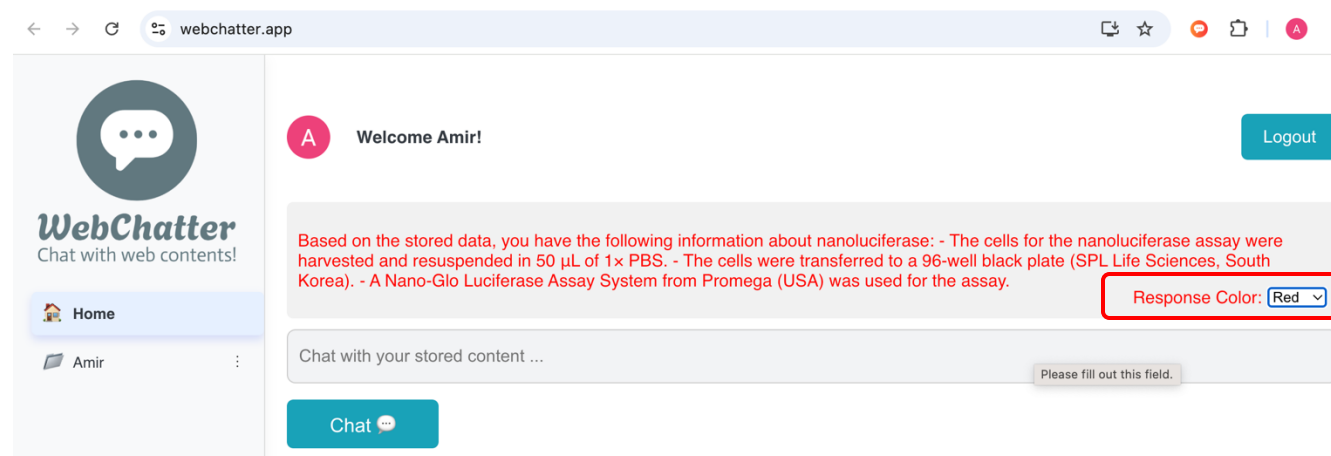
Based on the stored data, you have the following information about nanoluciferase: - The cells for the nanoluciferase assay were harvested and resuspended in 50 μ L of 1 \times PBS. - The cells were transferred to a 96-well black plate (SPL Life Sciences, South Korea). - A Nano-Glo Luciferase Assay System from Promega (USA) was used for the assay. Response Color: Black

Chat with your stored content ...

Please fill out this field.

Chat

Answers in desired appearance



The screenshot shows a web browser window with the address bar displaying "webchatter.app". The application interface includes a sidebar on the left with a "WebChatter" logo and a "Home" button. The main content area features a "Welcome Amir!" message, a "Logout" button, and a text block containing assay information. A dropdown menu labeled "Response Color:" is highlighted with a red box, showing "Red" as the selected option. Below the text block is a chat input field with a "Chat" button and a placeholder text "Please fill out this field."

webchatter.app

WebChatter
Chat with web contents!

Home

Amir

Welcome Amir!

Logout

Based on the stored data, you have the following information about nanoluciferase: - The cells for the nanoluciferase assay were harvested and resuspended in 50 μ L of 1 \times PBS. - The cells were transferred to a 96-well black plate (SPL Life Sciences, South Korea). - A Nano-Glo Luciferase Assay System from Promega (USA) was used for the assay.

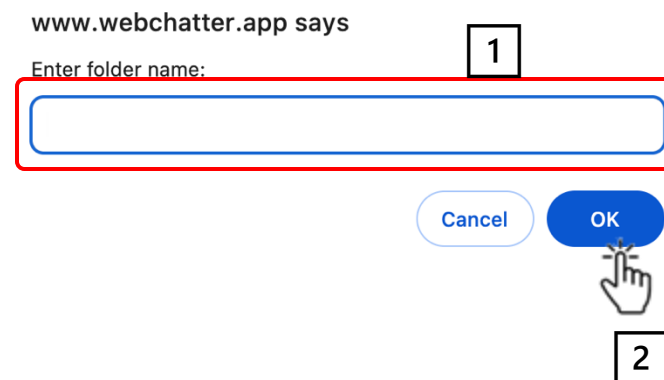
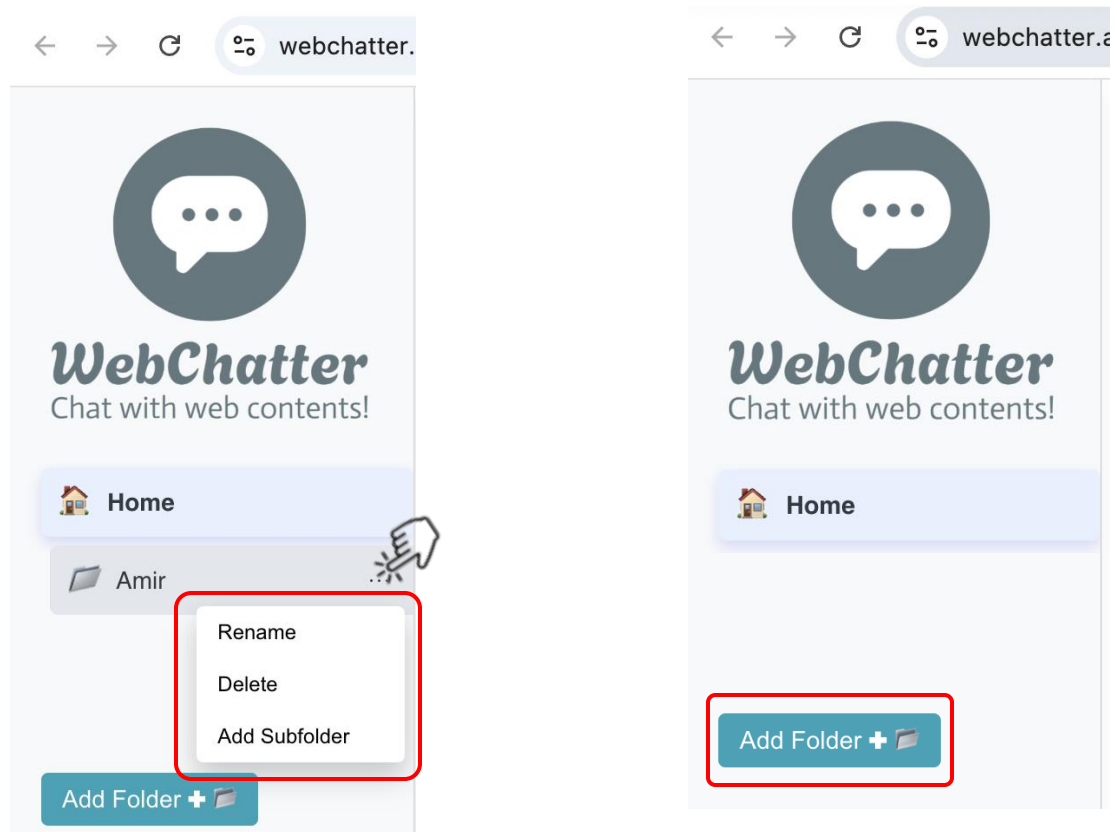
Response Color: Red

Chat with your stored content ...

Please fill out this field.

Chat

Manage Folders



Add new contents manually

Delete Selected

Add Content:

1

Lipofectamine (Endosomal) Method: Cells were transfected using Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific), following the manufacturer's instructions. RAW or dHL60 cells were transfected with 800 ng of nanoluciferase mRNA (the nonsecretory expressing form) in a 24-well plate with P3000 and reduced serum media, OptiMeM (Gibco, Thermo Fisher Scientific).

Add Content



2

Move contents between folders

Select	Content
<input type="checkbox"/>	<p>Nanoluciferase and Cypridina Luciferase Assay</p> <p>For nanoluciferase assay, the cells were harvested and resuspended in 50 μL of 1\times PBS and transferred to a 96-well black plate (SPL Life Sciences, South Korea). For the assay, a Nano-Glo Luciferase Assay System (Promega, USA) was used. See Less Delete Move to Folder</p>



1

Select a folder to move the content

Lipofectamine

Cancel

1

2

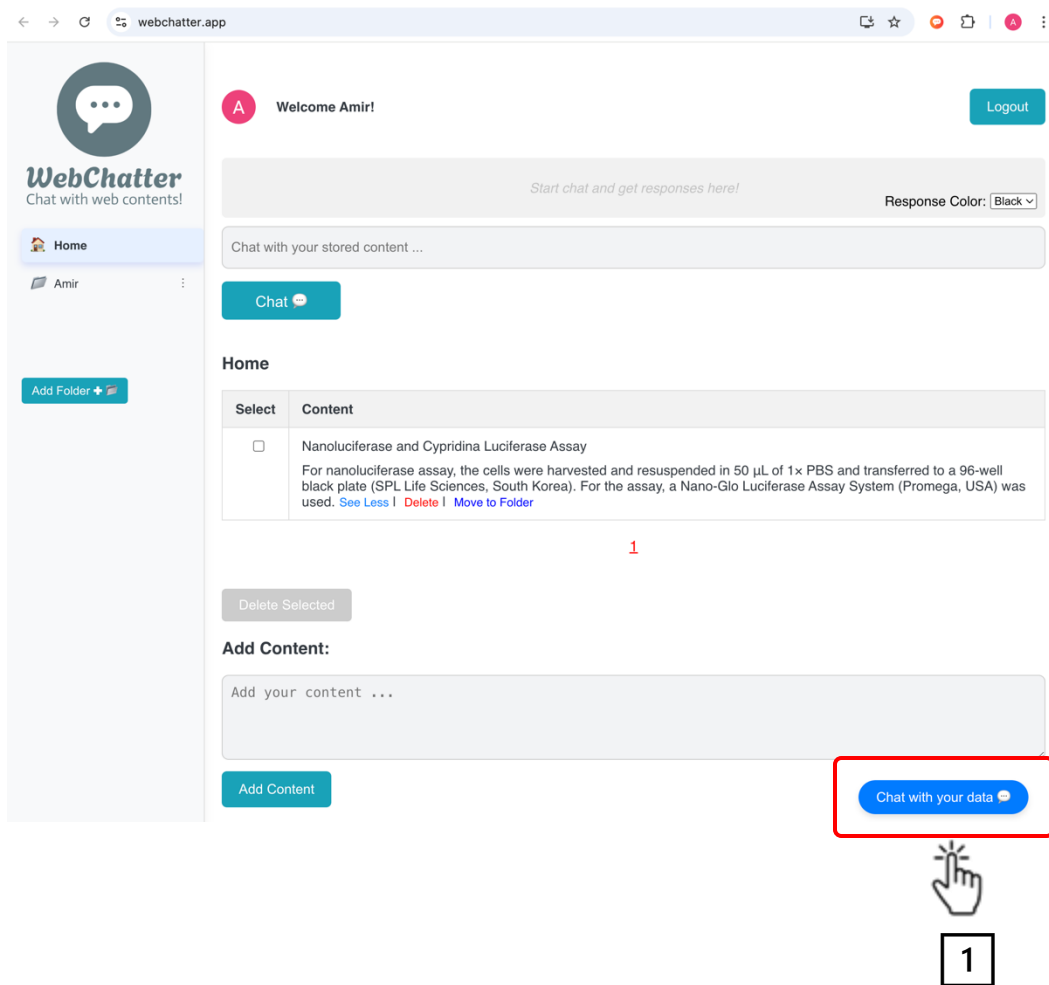
www.webchatter.app says

Content moved successfully!

3 OK



Chat with stored data: (2) popup chat



webchatter.app

Welcome Amir! Logout

Start chat and get responses here!

Response Color: Black

Chat with your stored content ...

Chat

Home

Add Folder

Select	Content
<input type="checkbox"/>	Nanoluciferase and Cypridina Luciferase Assay For nanoluciferase assay, the cells were harvested and resuspended in 50 μ L of 1x PBS and transferred to a 96-well black plate (SPL Life Sciences, South Korea). For the assay, a Nano-Glo Luciferase Assay System (Promega, USA) was used. See Less Delete Move to Folder

Delete Selected

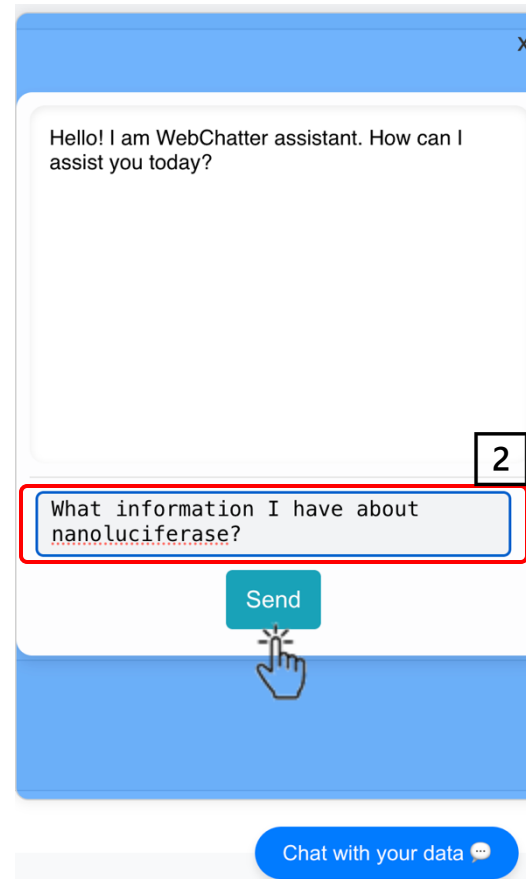
Add Content:

Add your content ...

Add Content

Chat with your data

1



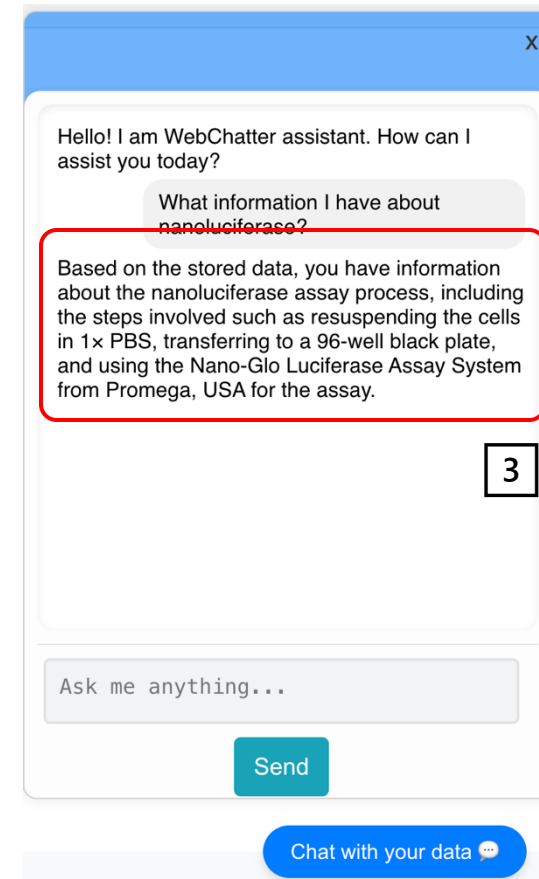
Hello! I am WebChatter assistant. How can I assist you today?

What information I have about nanoluciferase?

Send

2

Chat with your data



Hello! I am WebChatter assistant. How can I assist you today?

What information I have about nanoluciferase?

Based on the stored data, you have information about the nanoluciferase assay process, including the steps involved such as resuspending the cells in 1x PBS, transferring to a 96-well black plate, and using the Nano-Glo Luciferase Assay System from Promega, USA for the assay.

Ask me anything...

Send

3

Chat with your data