Universidade de São Paulo

Instituto de Química de São Carlos

SFI5869 - Técnicas de escrita científica em inglês

CORPUS

based on Folate Receptor, heterologous cloning and expression, and microfluidics papers

São Carlos
2013

SUMMARY

I. Explanation

II. Sample phrases

A. Abstract

a. *Context*
   - *declare topic's prominence*
   - *acquaint terms, processes*
   - *bringing on a technique*
   - *exposing an interesting feature of the topic/technique*

b. *Gap*
   - *quote problems*
   - *quote needings*
   - *quote gap/lack of previous research*

c. *Purpose*
   - *quote the main purpose*
   - *detail purpose*
   - *introduce purpose with materials and methods*
   - *introduce purpose with results*

d. *Materials and Methods*
   - *list the criteria and conditions*
   - *quote/describe materials and methods*
   - *justify the choice of materials and methods*

e. *Results*
   - *statement of results*

f. *Conclusion*
   - *exhibit conclusions*
   - *statement contributions of the work*
   - *statement of recommendations to future work*

B. Introduction

a. *Context*
   - *declare topic's prominence*
   - *acquaint terms, processes*

b. *Literature review*
   - *historical review of literature*
   - *current trends*
   - *progress in the area*
   - *state of art*
   - *previous author's work*

c. *Gap*
   - *unsolved conflicts*
   - *restriction of previous work*
   - *issues not yet considered*

d. *Purpose*
   - *State a new approach or methodology or technique*
   - *Explain purpose*
   - *Present improvements / advances of a topic in the literature*

- *Present an extension of a previous work of the author*
- *Specify the purpose*
- *Introduce more purposes*

e. *Materials and Methods*
- *list the criteria and conditions*
- *quote/describe materials and methods*
- *justify the choice of materials and methods*

f. *Results*
- *statement of results*
- *remark/discuss results and possible problems*

g. *Conclusion*
- *exhibit conclusions*
- *statement contributions/value of the work/research*
- *statement of recommendations to future work*

C. Materials and Methods

a. Materials
- *list, source, informations*

b. Methods
- *specific informations, justifications*

c. Facilities/equipment

d. Data analysis

D. Results

a. Rehash literature review

b. Rehash purpose

c. Rehash materials and methods

d. Results
- *exhibit*
- *discuss*
- *compare*
- *speculate*
- *explain*
- *exemplificate*

E. Conclusion

a. Context

b. Purpose

c. Materials and Methods

d. Results

e. Conclusion
- *present research limitations*
- *implications of the research*
- *make recommendations/future research*
- *present contributions / value of the research*

F. Acknowledgements

- support
- helpful discussions

G. Captions

III. Example phrases

I. Explanation

- Colors

The green marker simbolizes words that can be "easily"used.
The yellow marker simbolizes words that can be carefully used.
The purple marker simbolizes field specific words.
The red marker simbolizes tricky words, that should be avoided.

- Numbers

The numbers before the phrases refeers to the paper where it was found.

- Separation [1-5/6-10]

The articles 1-5 are based on Folate Receptor and heterologous cloning and expression, while articles 6-10 are based on microfluidic, different fields in wich my project in inserted. It is important to mention that, until this time, I have not worked with microfluidics, although this will be an important area in the project. So, I decided to prepare this corpus based on my entire master's degree.

- Papers/ Impact Factor

[1] Year 2011 IF: 3.888; 5-year IF: 3.592

Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 108 (2008) 619–626
Gynecologic Oncology
www.elsevier.com/locate/ygyno

Folate receptor alpha as a tumor target in epithelial ovarian cancer

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[2] Due 2013

Nuclear localization of folate receptor alpha: a new role as a transcription factor

SUBJECT AREAS:
CELL PROLIFERATION
CANCER STEM CELLS
DIAGNOSTIC MARKERS

Vanda Boshnjaku[1]*, Kyu-Won Shim[1,2]*, Takao Tsurubuchi[1,3], Shunsuke Ichi[1], Elise V. Szany[1], Guifa Xi[1], Barbara Mania-Farnell[4], David G. McLone[1], Tadanori Tomita[1] & C. Shekhar Mayanil[1]

[1]Department of Pediatric Neurosurgery, Ann and Robert H Lurie Children's Hospital of Chicago Research Center and Northwestern

[3] Year: 2011 IF: 4.897

Biochem. J. (1997) **327**, 759–764 (Printed in Great Britain)

Expression levels of functional folate receptors α and β are related to the number of N-glycosylated sites

Feng SHEN, Huiquan WANG, Xuan ZHENG and Manohar RATNAM[1]
Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH 43699-0008, U.S.A.

[4] Year: 2011 IF: 2.935; 5-year IF: 2.889

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Archives of Biochemistry and Biophysics 428 (2004) 64–72
ABB
www.elsevier.com/locate/yabbi

cDNA cloning, functional expression, and characterization of chicken sulfotransferases belonging to the SULT1B and SULT1C families

Lesley A. Wilson,[a,1] Geert E. Reyns,[b] Veerle M. Darras,[b] and Michael W.H. Coughtrie[a,*]

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Received 1 March 2004, and in revised form 10 May 2004

[5] Year 2011 IF: 3.891; 5-year IF: 4.260

Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 887–891
Journal of Nutritional Biochemistry

Cloning, yeast expression, purification and biological activity of a truncated form of the soybean 7S globulin α′ subunit involved in Hep G2 cell cholesterol homeostasis☆

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[6] Year: 2011 IF: 3.438; 5-year IF: 4.009

Polymer 51 (2010) 5883–5889
Contents lists available at ScienceDirect
Polymer
journal homepage: www.elsevier.com/locate/polymer

Feature Article

[7] Microfluidic fabrication of smart microgels from macromolecular precursors

Sebastian Seiffert*, David A. Weitz

Harvard University, School of Engineering and Applied Sciences, 58 Oxford Street, Cambridge, MA 02138, USA

Year: 2011 IF:

4.011; 5-year IF: 4.482

ARTICLE IN PRESS

Methods xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Review Article

Use of micro-emulsion technology for the directed evolution of antibodies

Diane L. Buhr [a], Felicity E. Acca [a], Erika G. Holland [a], Katie Johnson [a], Gail M. Maksymiuk [a], Ada Vaill [a], Brian K. Kay [b], David A. Weitz [c], Michael P. Weiner [a], Margaret M. Kiss [a,*]

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[c] Harvard University, 29 Oxford St., Cambridge, MA 02138, United States

[8] Year: 2011 IF: 8.349 (Small, Wiley)

full papers

Polymersomes

Polymersomes Containing a Hydrogel Network for High Stability and Controlled Release

Shin-Hyun Kim, Jin Woong Kim, Do-Hoon Kim, Sang-Hoon Han, and David A. Weitz**

[9] Year: 2011 IF: 4.186

Langmuir **2008,** *24,* 7651–7653

Double Emulsion Templated Monodisperse Phospholipid Vesicles

Ho Cheung Shum,† Daeyeon Lee,† Insun Yoon,§ Tom Kodger,† and David A. Weitz*

School of Engineering and Applied Sciences and Department of Physics, Harvard University, Cambridge, Massachusetts 02138

Received June 11, 2008

Ⓦ **This paper contains enhanced objects available on the Internet at http://pubs.acs.org/Langmuir.**

[10] Year: 2011 IF: 4.186

Langmuir 2003, *19,* 10281–10287

Spontaneous Formation of Lipid Structures at Oil/Water/Lipid Interfaces

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II. Resumed phrases

A. Abstract

a. ***Context***

- declare topic's prominence

[2] ... has traditionally been associated with...; more recent work suggests that ...

[5] ... previously demonstrated to ..., was cloned and expressed in...

[7] ..., are needed to ...

[8] ... are used to ... consisting of ...

- acquaint terms, processes

[1]....is a...that can be used as...

- bringing on a technique

[6] ... can be produced with ...;

[7] ... is a novel ... technology that utilizes ....

[7] Rather than ... allows us to ...

- literature

[3] In a previous study with ..., it was proposed that ... is required for ...

- exposing an interesting feature of the topic/technique

[2] ... more recent work suggests that it may also be involved in ...

- expose hypothesis

[7] Using this method, ...

b. ***Gap***

- quote problems in the area

[1] The goal of this study is to ... that lack specific information about ...

*[6] however, in existing methods, ..., because ....

*[6] This circumstance limits ...

- quote needings

[2] As ... expands, it also becomes more critical to understand ...

- quote gap/lack of previous research

[3] In a previous study with ..., it was proposed that ... is required for ...

[3]The ...

*[6] however, in existing methods, ..., because ...

*[6] This circumstance limits ...

- quote gap with hypothesis

[7] The use of ... has the promise of ... by permitting ...

c. ***Purpose***

- quote the main purpose

[2] In this work we examined the hypothesis that ...

[6] To overcome this limitation, we produce ...

[7] In this study, we demonstrate the ability of ...

[8] ... are used to ... consisting of ...

[9] We present a novel approach for ...using ...

[10] We report ...

- detail purpose

[7] In addition, we describe the application of ...

- introduce purpose with gap

[1] The goal of this study is to improve ... that lack specific information about ...

- introduce purpose with materials and methods

[3] The significance of ... was examined by ...

- introduce purpose with results

[4] A search of ... that appeared to ...

d. ***Materials and Methods***

- list the criteria and conditions [diferenca??]

[4] We cloned ... into ...

[4] Transformed ... strongly expressed ...

[4] Purification of ....from ... was accomplished by ... procedure involving ...

[5] The recombinant ...

[5] ... was purified by conventional biochemical techniques, and... was evaluated ...

- quote/describe materials and methods

[1] ... expression was analyzed by ... obtained from ...

[1] ... were also analyzed. ... was analyzed .....

[6] We use ... to ...

[8] To make ... are prepared ...

- justify the choice of materials and methods

[6] We use ... to ...

[8] ... dewets from ...; this dewetting leads to ...

[8] Subsequently, ...

[9] ... is used to ...

[10] We use ... to determine that ...

e. ***Results***

- statement of results /Describe/Discuss

[1] ...expression was apparent in ...

[1] ... was not associated with ...

[2] The work described here shows that ...

[3] In both ..., ... partly contributed to ...

[3] Further... resulted in the enhancement of...

[4] The ... displayed ... on SDS–PAGE, as predicted, ...

[4] Detailed ...analysis showed ...

[4] We also ..., which were able to ... which were able to ...

[5] ... were similar to ...

[5] The use of ... ruled out ...

[6] This approach ... and allows ..., thus enabling us to ....

[8] ... facilitates ... and increases ...through ....

[9] We show that ...

[10] We show that ...

f. ***Conclusion***

- exhibit conclusions

[1] FRα expression occurs ...

[3] The results indicate that ..., is critical for ... effcient folding and optimal expression of functional ...

- statement contributions of the work

[1] New findings from this study show that ..., suggesting that ... may hold promise for ....

[2] Until this time ... has only been considered as ..., these studies describe a novel ...

[4] This is the first detailed ..., and it demonstrates that ...

[5] These findings open new prospects in the studies aimed at identifying ..., as a gateway to ...

[6] In addition, ... allows us to ...

[8] In addition, this approach provides a ...

[9] Our ... technique is applicable to ...

- statement of recommendations to future work

[1] ... suggesting that ... may hold promise for ...

B. Introduction

a) ***Context***

- declare topic's prominence

[1] The...

[2] Traditionally ... has been associated with ...; however more recently... has been associated with...

[3] The ... is a ...

[3] The ...also ...

[5] The role of ... proteins in ... is a widely accepted issue.

[7] .., are commonly used to ...

[9] ..., also known as ...

[10] In some instances, however, ...

- introduce terms, processes obs: vem bastante com literatura

[1] While ... is unclear, in ... it serves as ...

[1] Unlike ..., ... allows ...

[4] ... is ... performs ...

[6] .... that are able to ....

[8] ... whose ...

[10] ... usually requires ...

b) ***Literature review***

- historical review of literature/background

[1] For example, ..., is currently undergoing ...

[3] ... have been identified ...

[5] In previous studies , ... was demonstrated ..., suggesting that ..., are likely to be produced by ...

[5] On the other hand, since ..., it seems unlikely that ...

[9] This approach has been applied to ...

[10] ... have been proposed to explain ...

[10] Alternatively, ... may be caused ...

[10] Finally, ...

- current trends

[1] This property has implications for ...

[1]...is an attractive candidate for ...

[6] A very common ...: it exhibits ... and has thus been used extensively to ...

[6] Since ... shifts upon ... can be controlled by ....

[7] Rather than ..., this method allows us to .... Depending on ...

[7] The use of ... has the promise of ...

[8] In addition, ..., which enables ...

[8] Recent advances in ... have enabled ...

[9] A new strategy to ...

[9] An example is the use of ...

[9] ... can be generated ...

[10] This is of great interest for ... and thus has stimulated ....

- progress in the area

[2] This study examines ...

[4] Recently, reports of ... have appeared.

[4] One of these is clearly related to ..., although ...

[4] It has also been shown that ... is carried out by ...

[7] Previously, ...i.e., ...

[7] This is in contrast to ...that involve ...

[7] Through the use of ...., it is possible to ...(i.e., ...).

[7] Thus, there has been a great deal of recent interest in ...

[7] ... is the most widely used method for ...

[8] ... can be used to ...

[9] Due to ..., these methods typically lead to ...

[9] Moreover, ...

[9] Since ... also provide ... for the study of ....

[10] ... has been reported for ... also known to ...

- state of art

[1] ...specific approaches have generated significant enthusiasm for ...

[6] It is this ... which makes them attractive for applications in .... such as ....

[6] A powerful method to ... is ...

[9] They are ... for biomedical applications.

- previous author's work

[2] Previous data from our lab demonstrated that ...

[5] From these statements, our strategy was to test the effectiveness of ... by ...

[7] We have previously described a novel approach that utilizes ... for ...

[7] The ... has been applied to the ...and for the ....

[7] Using the method we developed , ...

c) ***Gap***

- unsolved conflicts

[1] What is unknown in attempting to ..., the ...of ... and whether ... is (lost) or (maintained) with...

[2] A ... mechanism of ... may be through ... and acting as a ....

[3] It has been demonstrated that ... does not affect the ability of ....

[3] In contrast, ..., it was demonstrated that ... is essential for the ...

[6] However, the ... behavior also depends on ... such as the ... and ...; it is therefore crucial to control both the ... and ... to design ... with ...

- restriction of previous work

[1] In addition, although ... has stated that ... is an indicator of ..., the ...was ...

[2] ...and colleagues reported the presence of ... on ..., ; however the relevance of ... was not determined.

[5] There is a paucity of information on ..., however.

[5] ...have a number of ... that are related to .... and are involved in ... but, to date, there is little information on ....

[8] However, despite ..., the use of ... is limited due to ..., which leads ...

[8] Moreover, ..., making it difficult to ...

[10] When ... is placed in contact with ..., ... can form; however, it is unclear whether ...or... occurs.

- issues not yet considered

[9] Another strategy is to ...

[9] However, it is difficult to precisely control ....

[10] By contrast, to our knowledge, no observations of ... have been reported.

[10] Thus, ... is still ...

d) ***Purpose***

- State a new approach or methodology or technique

[4] Since ..., is an important pathway of ..., we were interested to discover whether ... also possesses ... with the capacity to ....

[6] One idea to control ...is to use ...; the key element of this strategy is to control ...by subsequent ...

[8] In this paper, we report ... approach to make ...; the ... provides ...

[9] Here, we present a novel technique for ... using ...

- Explain purpose

[3] The present study was undertaken to identify ... to test the role of ... in ...

[3] Our working hypothesis was that ... is either the most significant or the only contributor to ...

[9] Because of the resemblance of ... to ..., ...that rely on ... should be ...

- Present improvements / advances of a topic in the literature

[2] In this work we tested the hypothesis that in response to ..., ... and acts as ...

- Present an extension of a previous work of the author

[5] For this reason, our research has been directed to seeking ... responsible for the...

[5] On the other hand, since ..., we decided to develop..., aimed at identifying ...

[7] In this study, we demonstrate the application of ...

- Specify the purpose

[1] We performed this study to define... and to determine whether ... was associated with ...and ....

[6] In this paper, we review our work on the use of ... to produce ...

[10] In this paper, we study ... (phenomena) occurring ... when ....

- Introduce more purposes

[1] In addition, we examined ... as well as ...

- Introduce the purpose with results

e) ***Materials and Methods***

- list the criteria and conditions

[8] Using ..., we prepare ... which are... through ...

- justify the choice of materials and methods

[3] To address these issues, we used a ... approach to either eliminate or introduce ...

[3] We studied the effects of these changes on ..., their ability to ... and the extent of ...

[5] To this purpose, ..., which will be referred to as ... in this article, was undertaken.

[9] In our approach, ...

f) ***Results***

- statement of results

[5] The objective was achieved ...

[5] The...was purified and its biological activity assessed in ...

[5] These data suggested ... for the development of ...

[8] Upon ..., the ... are ..., resulting in ...

- remark/discuss results and possible problems

[4] This represented ...

[4] The recombinant ... was able to ..., but no more information about ... is available.

[5] The results of these studies pointed out that ... could be induced in ..., as well as ...

[8] The .... facilitates ... owing to ...

[8] In addition, ..., preventing ...; this leads to ....

[9] Such ... have previously been used as ...; however, in our work, we use (them as) ..., as illustrated in Scheme 1.

[9] However, ...; thus, this ...

g) *Conclusion*

- exhibit conclusions

**[4] As a first step towards determining ...., we report here the cloning, sequence analysis, expression, purification, and characterization of ....

**[5] By the use of this .... approach, (adequate amounts of the recombinant) ... could be obtained and tested ..., as a first step to further ....

[8] Moreover, we demonstrate that ...provide ...

[9] Here, we introduce new strategies to improve the....

- statement contributions/value of the work/research

[9] Our technique can be used to create ...

C. Materials and Methods

a) Materials

- list, source, informations

[1] ... specimens from ... were eligible for inclusion in the study.

[3] All of the ...were constructed by using PCR.

[4] ... were purchased from ... (city, country).

[5] ... strain ...was used for ... expression.

[5] The ... strain utilized for genetic manipulations was ...

[5] Restriction enzymes ...and ... were purchased from ...

[5] ...was purchased from ... (..provided, we bought ...)

[5] The ... for ...were obtained from ...

[5] Other chemicals were ....

[7] The ..., was generated by ... provided by ... amplified by ....

[7] The ...reaction contained ...

[8] To make ..., we use ....

[9] The ... was made of ...

[9] Unless otherwise noted, the ... consisted of ...

[9] Experiments were conducted with ...

[9] All ...were purchased ...

[9] The ... was either ...or ...

[9] Solutions and solvents were all filtered before ...

[9] ... was acquired from ...

[10] The ... used in these experiments was ... . was used to prepare ...

[10] A ... was prepared by ...; subsequently ...

[10] ... was prepared by ...; the addition of ... prevents ...

[10] Finally, ... was used for the ...experiments. The ... and ... were used, without further purification, within ...months of purchase.

[10] These ... were stored in ... and kept in a freezer at X °C ...

[10] To prepare ... suspension used for ..., we first ...

[10] After ...was formed, ... was added to ..., and ... was achieved by sonicating ...

[10] Finally, ...

b) Methods

- specific information, justifications

[1] .... were selected prior to ....

[1] The ...chosen for the study were (similar to)...

[1] There was a difference between ...

[1] This was caused by ..., which resulted in ...

[1] ... were reviewed by ...to confirm ....

[1] ....were examined for ... expression by assessing triplicate ...

[2] To test the role of ...as a ... we examined ...

[2] These ... were chosen because in our previous studies, working with ..., we found that ....

[3] The resulting ... were digested at ... and substituted for .... in the plasmid ...

[3] ..., were used in combination with....containing ...

[3] The recombinant plasmids were amplified in ... and the ... sequence was verified by ...sequencing with ...

[3] ... were grown in ... medium ... supplemented with ... . were performed with ... in accordance with...

[3] Cells ...were washed in ..., twice with ..., and once again with ...

[3] The cells were then incubated with ...for...

[3] After ..., ...was recovered in ... and subjected to ...

[3] The specific ... was determined by ..., which had been ...

[3] The specificity of ... was also established by ...

[3] ... were dissolved in ... and insoluble material was removed by centrifugation at X***g*** for Xmin.

[3] Simultaneously, ... was added to a final concentration of X%.

[3] The ...were incubated with ...;... was assayed by ...

[3] ... were accomplished by ...

[3] Insoluble material was removed by centrifugation at X ***g*** for X min.

[4] The ... assay was used to ...

[4] Protein, incubation time, and substrate concentrations were optimized for ...

[4] ... were assayed in (duplicate) with a control ...containing ... in place of ...

[4] Reaction mixtures comprised ...

[4] The reactions were incubated for X min at X °C before being stopped by ....

[4] This was then followed by the addition of ... causing ...

[4] The ...were then centrifuged at Xg for X min.

[4] ... of the supernatant was then mixed with ... and ... was quantified by ...

[4] For ... was measured using..

[4] The assay buffer was ...(pH...)

[4] The... were incubated at X °C for X min before ...

[4] ... were measured essentially as described by ..et al.

[4] Fractions were collected, and ... was corrected for ...

[4] For determination of kinetic parameters, ...were carried out to determine ..

[4] Assays were performed over ..., and ... were estimated using ...

[4] Following sequence analysis, we designed PCR primers to amplify the predicted coding regions of ... to facilitate ...into either ... or ... for expression in ...

[4] PCR was performed as previously described, using ...

[4] Overnight cultures were used to inoculate ... supplemented with ...

[4] Expression was induced, once ... was reached by...

[4] The ...were then incubated for approximately X h at X °C, with shaking at Xrpm.

[4]... was produced by centrifuging ... at Xg for X min at X °C.

[4] The supernatant was then decanted and the pellets were ...

[4] After lysis of the cells, the ....was resuspended in ..., and centrifuged at Xg for X min at X °C.

[4] The ... was used to ...exactly as described previously, using a....

[4] For immunoinhibition experiments, E. coli cell-free extracts (prepared from ...) were first incubated with ....

[4] ... were estimated by the methods of ..., using ... as standard.

[4] ... were resolved on denaturing SDS–PAGE (12.5% acrylamide monomer), and visualized by Coomassie blue staining.

[5] .... was cultured in ... medium ...

[5] The... transformants were selected in ...

[5] All ... cultures were maintained at X°C.

[5] For..., ... were cultured to an optical density of ...

[5] Then methanol was added to ....

[5] All ... transformants were selected in ...

[5] The ... gene was amplified by ...

[5] The oligonucleotide ... was designed to ...

[5]A second oligonucleotide ...was designed to generate

[5] Both ...were dissolved in ...

[5] The ... mixture consisted of ...

[5] PCR amplification was carried out on ... using the following conditions: ...

[5] The PCR X bp product was cloned into ...and transformed in ...

[5] Positive clones were selected on ....

[5] One of these clones was sequenced ...to ensure that ...

[5] ... were linearized by ... with ... and then purified.

[5] ... were transformed by electroporation with ...

[5] Transformants were first selected by ...

[5] In order to verify ..., the ... was used to ...

[5]...wass used as template to verify ...

[5] ... was performed using the following conditions: ....

[5] ... were grown in ....

[5] The inducing phase was triggered by adding ...

[5] ...were examined for expression of ...

[5] The .. was selected for .... and grown under ... conditions ....

[5] About ... was centrifuged at X×g and X°C for X min with ...

[5] The ...contained in the ...were precipitated by ...

[5] The ...was dissolved in ... and precipitated by ...

[5] The ...was centrifuged for ....

[5] The ... was dissolved in ... and loaded on ...equilibrated with ...

[5] The ... was carried out with ..., respectively.

[5] The fraction eluted with ... displayed ...

[5] ... under ...conditions ... was carried out on ... using ...

[5] The gels were stained with ...

[5] In order to assess ... were tested by ..., essentially as described in Ref. X.

[5] ... was determined by ..., using a ....

[5] Differences in ... were determined by ...followed by ....

[5]... are expressed as ....; ... were considered as ...

[6] We fabricate ..., followed by ..., as illustrated in Fig. 1.

[6] Using ... allows us to obtain ....

[6] Due to ... achieved through ..., these ... are ..., and ...

[6] In addition, this approach allows us to .... such as ...

[6] To form ..., we use ... based on ..., as shown in Fig. X

[6] For this purpose, we ...

[7] The ... were generated by ... provided by ...

[7]... displaying .... were incubated with ...and emulsified with ...

[7] After overnight incubation with shaking atX °C, ...

[7] The...were incubated for ....

[7] The ....was digested with ... and ligated into ....

[7] ... were prepared by ...

[7] ... were coated with ...

[7] After rigorous washing with ..., ... were eluted by ...

[7]... was carried out for X min. at X °C without shaking.

[7] The following day, ...were scraped into ... containing ... .was added to a final concentration of .... and ... stored at X °C.

[7] The... were held at X °C ... and then centrifuged at Xx g for X min.

[7] The... was vortexed at ... to ...

[7] ... was estimated to be ...

[7] The ... were incubated in a X °C shaker overnight for ...

[7] After overnight incubation, ...

[7] Once the OD600 reached X, ...

[7] After..., ... were centrifuged at ...

[7] The ... were expressed from ... in ... supplemented with ...

[7] ... were used to ensure ...

[7] The cultures were centrifuged and the pellets frozen at −80 °C.

[7] Cell pellets were resuspended .... supplemented with ....

[7] The...were incubated with ... and then sonicated Xx for X s each (with a X min pause between each pulse).

[7]... were clarified by centrifugation at X rpm in a X rotor for X min at X°C.

[7] The ... were purified from ...

[7] After ..., ... were washed with ...

[7] Eluted protein was collected and analyzed by SDS–PAGE. [we... using ..technique]

[7] The column was equilibrated with ..., pH ...

[7] The ...was bound to ...and eluted with a gradient of ...

[7] .... was conducted on ... to determine the amount of ....

[7] The .... were exposed to .... and then allowed to ...

[7] ... were ... followed by ....

[7] ... was detected using ...

[7] To test ...were ....

[7] ... was detected using ...

[7] For kinetic analysis, ... was coupled to ...

[8] To control .... we use ...

[9] ... were prepared in ..., which have been described previously.

[9] The ..., were purchased from .... and tapered to ...

[9] These ... were controlled by ...

[9] A typical ...

[9] The ... was monitored with ...

[10] The ... was measured using ...

[10] The ... depends on ....

[10] For ... where ... are valid, ... to determine ....

[10] This method has been used for ...

[10] In the experiments reported here, ... was measured by ...

[10] Both ...and ... were acquired to determine the nature of .....

[10] Briefly, ... provides .... based on ....

[10] Because ...,... is generated ..., which permits...

c) Facilities/equipment

[3] The reactions were performed with ...

[4] Sequencing of clones and expression constructs was performed on ....

[9] ... were obtained with .... equipped with ...

[9] The process of ...were imaged with ...

[10] The .... was monitored and imaged .... The ... was determined and ... was .... by the ...

[10] .... were carried out with ...

[10] The composition of ... was determined using ...

[10] In our experiment, ... were acquired by ...

[10] To study ...,...was formed by ....

[10] .... was used to determine ....

d) Data analysis

[7] Data was analyzed by ...

[10] The distributions of ...., were mapped by ....

[10] ... was performed on ...

[10] ...., this analysis provides a reasonable estimate, but ...was used to determine ...

D. Results

a) Rehash literature review

[7] We have previously described a novel approach that utilizes .... for the identification and isolation of ...

[8] .... provide a convenient method to make ...; this is accomplished by employing ...

b) Rehash purpose

[2] To test the hypothesis that ... was performed in ...

[3] To identify ...

[6] For this purpose, it is advantageous to employ a ... which allows ...

[7] In the present study, we explore the potential of this technology for ....as well as ...

[8] By contrast, our approach enables ....; if necessary, ... can be removed by ....

[8] Therefore, this approach is potentially useful for ...; such .... have potential value for ...

[9] ... are generated with ....shown in Figure 1a.

c) Rehash materials and methods

[1] Because of ...., we compared ... to ...

[2] The ratio of .... were determined using ...

[2] To determine whether ....

[2] To confirm .... experiments were performed using ...., an area where both of ...

[3] Before the effects of .... were tested, it was desirable to ensure that ...

[3] Therefore .... were synthesized by ...., analysed on .... and detected by ....(Figure X)

[4] By ...., we identified .... which appeared to ....

[4] First, we completely .... and the ....data are shown in Fig. X.

[5] As mentioned in Materials and methods, the only difference between ....which, for technical

reasons, was ...

[5] .... were collected and analysed by SDS-PAGE (Fig. 3).

[7] To test the hypothesis that .... could be used to ...., we produced ....

[7] After overnight incubation of ...

[7] The .... were propagated and analyzed by ...

[8] The .... is comprised of ...., as shown in Figure 1.

[8] One .... is tapered to .... and is treated ....; this is used for ..... The other ....

[8] As an ...., we use ....; we employ .... to study ...

[8] We inject ....

[9] The ....is an ...., while the .... is an ....

[9] The overall .... can be adjusted by ....

[10] The ...., was measured for ....

[10] Using ...., we ...

[10] We investigated ...

d) Results

- exhibit

[1] The primary ....analyzed in this .... are representative of ...

[1] .... were more likely to be .... than ....

[1] .... staining was evident in ....

[1] Specifically, of the ..., there was strong staining in ...., ...and...., but no staining in ....

[2] The results of .... using ....(Fig. X) showed that ...

[2] The results confirm ...

[3] ...., showed multiple or diffuse bands, as expected, ...and the presence of ....

[3] Both the .... and the .... showed bands of similar intensities (Figure X), indicating ....

[4] .... analysis of ... (Fig. X) indicated that ....

[4] Examination of the .... showed they both ...., in particular .....

[4] Pilot experiments with .... showed that .... were able to ....

[4] We demonstrated the presence of ...., and we are currently performing ....

[5] The .... are shown in Fig. X, respectively. We show in figure 1.....

[5] Fig. 2 shows ...., to verify ....

[5] The size of ...., thus indicating that ...

[5] The ... was achieved by ....

[5] Lanes X and X of Fig. X show the electrophoretic patterns of the ..., respectively.

[5] As it is shown, a faint band at X kDa, indicating the expression of the ...., was detected in ....

[5] The effect of .... is shown in Fig. 3.

[6] Working with .... can be formed ...., whereas .... ensures that ....

[6] If ...., whereas ....is achieved when .....

[6] After ...., ... are obtained by ...., achieved through .... (Fig. 1).

[7] Consequently, ... are produced, some of which ...

[7] ....analysis of ... indicates that it has a .....

[7] The majority of .... showed ...., indicating that ...

[7] We show here that we can use ....

[7] In addition, we show that .... can be used to ....

[8] Therefore, ...

[8] ... ; this ... is caused by ....

[8] .... induces ....and, ultimately, ... as shown in Figures XX.

[8] Once ...., ...

[9] The ...., shown in Figure X, makes them ideal .... for ....

[9] .... are obtained from .... (Scheme X).

[10] Figure 1 shows the results plotted as ...., as suggested by X.

[10] .... revealed the presence of ...., which were determined to be ....

[10] Both of .... were formed by ....

[10] These results are plotted in Figure X.

[10] In Figure 3B, ... represent ....

[10] .... reveals the presence of ....

[10] At ...., we observe ....

[10] We found that .....

[10] We did not observe any ....

[10] Instead, we saw ...., as shown in Figure X.

[10] We observed that .....

[10] In addition to ...,... were also observed ..., as shown in Figure X, ...

[10] This behavior was observed ...

[10] We have observed that ....

- discuss

[1] Therefore, ...

[1] Interestingly, ...

[2] It is to be noted that ..... was present in ... even in the absence ...

[2] It is to be noted that .... used here also showed ...

[2] In the absence of...., ... whereas in the presence of ...., ... appeared to ....

[2] When the data in Fig. X is presented ....,.... is present in ...., there is a significant increase in ....

[2] This demonstrates that ....

[2] These data indicate that ... is not limited to ....

[3] A similar analysis of .... revealed ...., ... which corresponded to ....

[3] Treatment of .... resulted primarily in .... corresponding to ....(Figure X)

[3] It is concluded that .... are both ....

[3] In general, ... showed progressively .... (Table X).

[4] It is interesting that .....

[4] This was somewhat surprising as ....

[5] A relevant .... was already achieved with ...., but ....and allowed ....

[6] The use of .... provides a very useful benefit: since ...

[6] Apart from .... can also serve to ...

[6] By this means, the use of .... allows not only ..., but can also serve to ...

[6] By contrast, ..., thereby providing ...

[6] Thus, when ....

[6] Upon ...

[6] Then, ... can be removed and .... can be stored at ....

[7] We believe that .... provides numerous, significant advantages over ....

[7] For one, by ...

[7] Second, ... can be reduced to ...

[7] Reducing .... not only ...., but it also ....

[7] In a typical ...., it is difficult to .... due to ...

[7] Because ....are no longer...

[7] Another clear advantage of .... is that the technique can permit ....

[7] Generally, ...

[7] Using .... we can apply ...., enabling ....as has been achieved with ....

[8] Therefore, ...,... as schematically illustrated in steps X of Figure X.

[8] Subsequently, ..., as schematically illustrated in steps X of Figure X.

[8] After ....; this leads the formation of ...., as schematically illustrated in steps X of Figure X.

[8] Although ...., the .... is not significant because ...; thus, ...

[8] Nevertheless, ..., as shown in the .... images; ...., while approximately ....

[8] .... which exhibit ..., while .... which have .... allow the ....

[8] When ...., their... due to ....; nevertheless, there is no ....

[8] However, ...

[8] This occurs ....., as shown ... images in Figure X; ....

[8] Although ...; we estimate ...as approximately X.

[8] By ..., we clearly observe ...., as shown by .... in Figure X.

[9] In the absence of ..., ... suggesting that ...

[9] The reproducibility of the technique is further improved by carrying out ...

[9] When ...., they can become ...., and...

[9] Upon ...., as shown in the .... of Figure X.

[9]This process resembles a method where .... are formed in ...., resulting in ....

[9] In our case, ...

[9] Using the same approach, ...have been generated using a variety of ....

[10] However, ..., indicating a slower/higher decrease/increase in .... is not reached .....

[10] This change in .....is reproducible, ruling out .... such as .....

[10] However, ... reported for ...., suggesting that ... does not result from ....

[10] For these experiments, ...was dispersed ...

[10] Since these experiments were...

[10] We measured ...

[10] In addition, we used ... to measure the time evolution of the ....; the results are plotted in Figure X.

[10] The ..., which determines ...., and hence ...; as the ...gets thicker, the ... get larger.

- compare

[1] Similarly, when .... were grouped with the ... in order to assess the effect of ...., no association could be found between ...to .... (data not shown).

[1] This is consistent with the effect of ...

[3] Similarly, ... resulted in ...

[3] In the absence of ... was expressed .... compared with ...

[4] This is different from ..., since ...

[4] Similarly, ...

[4] Therefore, ... towards .. are broadly similar to ....

[5] The ... produced a significant rise/decrease in ... compared to ...

[5] The result obtained with ...was similar to that of ...

[5] At ...was there any evidence of ..., as determined by the ...and ... assays (not shown).

[6] One of them uses ...., and the other one uses ...

[8] .... compared to that ..., the ... decreases/ increases, thereby reducing/increasing ....

[8] By contrast, under the same conditions, ..., exhibit ..., thereby ..., as shown in Figure X.

[8] By contrast, the ..., as shown in the ...of Figure X.

[8] For comparison, we determine the ...dependence of the ... of the... using ..., where ... of Figure X.

[8] ... is more effective when the ...

[9] Such a ... phenomenon has also been observed when .... are used for ....

[10] For times shorter than X min, ...; this ...dependence is consistent with ...

[10] When ..., these ... are clearly ...and have ..., consistent with previous observations of ...

[10] Though ... was more apparent at ..., closer inspection reveals that ...

[10] In general, ...formed with... are smaller than ....that does (not) change significantly as we ....

[10] By contrast, ... are ....

- speculate

[2] These results suggest the following: (i) In the absence of .., there is ...; (ii) Upon ... treatment, ...is...; (iii) Of the (two) .... bands, the .... seems to ...

[2] The above studies suggested that ..., it is enriched in ...

[3] This result suggests that .... is not dependent on the presence/absence of ... but is instead dependent on the expression level of ...

[3] From Tables X and X it is clear that ... (Figure X) is not the only ..

[3] Although it seems that in the absence/presence of ....., there is a greater decrease/increase in the expression of....., it might be concluded that, in general, every... contributes to the ....expression of ...

[4] ...this sequence contains ... that is believed to play a critical role in ....

[7] This data suggests that ... is sensitive enough to ...

[7] By varying ...., such as ..., it may be possible to ...

[7] We can also ..., since differences in ... could influence ...

[8] We attribute this ...decrease/increase to the ... by comparison with that ...; because of ...

[8] We attribute this increased/decreased stability of ... to the formation of ... which supports ...; this prevents ..., ensuring .... is maintained, as shown in Figures X.

[9] We believe ... plays an important role in ....;however, the exact ....mechanism is yet to be established.

[10] This suggests that ...

[10] These results provide strong evidence that ... does (not) affect the ...

[10] This strongly suggests that ... results from the presence of ....

- explain

[1] ... showed ... based on ..., although there was a modest trend towards ... that was not reflected in ... (Fig. X).

[4] We therefore propose that this ... be named ...

[4] To study the properties of ..., we expressed the .... in .... as previously described for ....

[4] The data presented here suggest ...., a conclusion also supported by the ....analysis (Fig. X).

[6] Both techniques offer versatile means to ..., and.... opens a route to ...such as ....

[6] We use ....to fabricate ... from .... which consist of ...

[6] In addition, this strategy can be extended to .... by the use of ...

[6] To demonstrate this concept, we synthesize ... which contain ....

[6] These .... are useful to ....; in a typical experiment, we use them to ...

[6] For this purpose, we use .... to form ..

[6] To circumvent this limitation, we employ a ... approach: first, we create ... which serve as ...

[6] In the first .... we add a ... as the .....

[6] In the second ... we add ...to form ....

[6] Due to this ...., these ... are applicable for ...purposes: when ...is... it is ..., whereas it becomes ... when ...

[7] To determine whether ... technology could also be useful for ...., we first investigated ....

[7] After ..., the... were tested for ... by selection of ...

[7] In fact, our data indicates that ... remains ....after ....

[7] Because ....

[7] This application relies on the... resistance of the target protein or peptide.

[8] To explore the ... of the ..., we investigate their behavior when exposed to ....

[8] As the ....is lowered/increased from .. to ..., the ...of the .... increases/decreases owing to ... caused by the ..., as shown in Figures XX.

[8] Due to ..., the ...are...., finally, ...; this results in ...., as shown in Figure X.

[8] The .... can also be enhanced by ...

[8] We summarize these ... different ...behaviors schematically in Figure X.

[8] We illustrate this by ....in Figure X.

[8] This ... is a result of ..., which limits ....

[8] Therefore, ...

[8] ...can ..., thereby preventing ...

[8] ...; we attribute this to .... than ...

[9] We use a mixture of ..., to facilitate ....

[9] As ...., the.., thereby ...

[9] At the later stage of ..., as shown in ..

[9] Due to ..., the...

[9] To avoid this, we find that .... is critical; thus, we use ...

[9] .... can also be formed through ...

[9] This ...route to ... offers a simple and effective way of obtaining ....

[9] To demonstrate ... of our approach, we encapsulate ....which are labeled with ...

[10] To examine whether ...., we prepared ...: one with ... and the other with ...., which prevents ...

[10] To monitor the appearance of ..., we repeated ...and used ... to measure ....

[10] To investigate the impact of ..., we imaged ....

[10] To investigate the .... involved in the ... process, we studied ....

[10] To elucidate the influence of .... properties on the ...process, we measured ....

[10] More information about the ... can be obtained by examining.... that are produced from...

[10] To investigate the differences in ..., we used ...

- exemplificate

[4] For example, ..., which contains ...., is located towards the N/O-terminus of .., and has a consensus sequence of ...

[4] For example, in... ,whereas the..., and ...are...

[6] In an illustrative example, we form ... which are ...and exhibit ...

[8] These...can...; for example, ...are shown in ... images in Figures XX

[8] In this case, ... has very little/big influence on ...; for example, ... which are .... exhibit ... as shown in Figures X

[8] For example, when ...., made by ..., are dispersed in ...., they remain as ...and then ...., as shown in Figures X

E. Conclusion

a) ***Context***

Literature review

[1] Other groups have reported using ... assays to analyze ...

[2] Previous work from our lab demonstrated that in the absence of ..., ... increased, through .... This ... affected ...

[2] ...and colleagues compared levels of .... and assessed differences in..., from ....

[2] ... was significantly lower/higher in ..., whereas ... was significantly higher/lower, possibly due to enhanced/decrease expression of .....

[2] ...and colleagues reported that whereas ...was...

[5] The ... has been demonstrated clearly.

[5] ... is currently the most potent .... tool for ..., thus providing a unique opportunity for....

[5] Moreover, it is clearly established that .... is greater in ...

[5] The hypothesis that .... arose from experimental studies indicating that ...., as well as ...

[5] To identify ..., were carried out with....

[5] We concluded that ... and confirmed this finding in/by....

[5] Although these data support the hypothesis that ... is responsible for the ..., arguments may be raised in favor of ..., since ....

[5] However, an increasing number of ... are being claimed to play relevant ....

[5] As far as ... is concerned, experimental evidence clearly indicates the possibility that ...may be..., thus eliciting a number of effects, including ...

[5] Other .... have been shown to exert .... effects.

[5] Recently, ..., has been identified from ....

[5] In this case, an increased/decreased ... was detected in .... Other .... have been shown to exert ....

b) *Purpose*

[1]The strengths of our study include the use of ...

[2] In this study we have elucidated a ... mechanism for ..., through ...

Purpose with Results

[4] Here, we report the first detailed ... analysis of .. from the ...

[4] The data presented on these ... clearly show that ... are closely related ..., and we have produced ... with which to investigate further their role in ....

[5] Therefore, in the search for ..., we described in the present work the cloning, yeast expression and purification of a recombinant ... which contained the ...and proved to be even more effective on ...

[8] In this work, we report a method to produce ...; these provide enhanced ...

[10] We have shown that when ... is placed in contact with ...., ...occurs ..., creating ....

c) *Materials and Methods*

[1] Another strength is .... due to ... of the study.

[1] We utilized ..., which has ...

[8] .... are prepared from ...;

d) ***Results and discussion***

[1] Nevertheless, studies assessing ... such as ..., including ...., may have different results.

[2] This ... does not take into account ... and it is still unclear exactly how...

[5] The present results suggest that ....

[5] Moreover, we found that ....

[6] The combination of .... with subsequent .... offers promising means to form ...

[8] ...subsequent .... creates a ....

[8] Accordingly, ... can .... in a more safe and precise fashion, preventing undesired ...

[8] In addtition, ...

[10] The ... explains the ....

[10] It also accounts for the ... produced by ...

[10] In addition to ..., we have observed ...

[10] We have identified the mechanism as being due to ,,,

[10] ...., which seems to be responsible for the formation of ... observed in ....

e) ***Conclusion***

- present research limitations

[1] A limitation to the study is that ...., especially in .... that are most likely to ....

[1] In addition, because ..., this ... study cannot be used to..; in other words, our observed proportion of... is likely an....

[5] How this ....could be achieved ....is difficult to establish as yet and this will require further investigations.

[5] However, ... has been shown.

[6]However, despite ..., these existing techniques of ... have an intrinsic limitation: since ...., ...is limited.

- implications of the research

[1] In summary, the common... as well as .... suggests that ... may be helpful for ..., whether...

[1] A number of strategies to take advantage of this observation are being pursued, with ...currently undergoing testing in ...

[2] This novel role of ... as a .... provides insight into ...

[2] It also provides ... to explore for treatment of diseases associated with ...,

[3] It should be noted that, for many ...., there are no practical means of ...

[3] An obvious implication of this finding is that .... is (not) likely to be involved in ... but, rather, ...might ..., either by ... or by ...

[5] This finding might explain ..

[5] The data obtained in the present study are intriguing because they show for the first time that... that are similar to those reported ....

[5] Moreover, the use of a recombinant protein rules out ..., for which ... have (not) been claimed.

[6] This approach separates ... from ... and allows ...; it thus combines the ... with the ...

[7] We have shown that ...

[8] Therefore, this novel approach to make a ... will provide new oppotunities for ...

[9] In conclusion, we present a general method for ...using ...

[10] The study of ... shows that variations in ... are related to changes in ...due to...

- make recommendations/future research

[1] Prospective studies of ... and its association with ...would be required to confirm these results.

[2] However, ... suggests that ...may also have a role .... Future work shall test this hypothesis.

[2] Further work needs to be done to examine ... and its' role in ...

[3] Further, the effects of this...can be studied.

[3] These observations, together with the finding that .... can be further enhanced by ..., provide

strong evidence that ... that is critical for ....

[5] Studies are in progress to identify ...., and to trace ...

[5] In addition, in order to reveal .... properties of the recombinant ..., experiments will be performed ...

- present contributions / value of the research

[2] The observation that ... is relevant to our understanding of the mechanisms of ... and has significant implications for ...

[2] The data presented in this paper provides relevant insight to ...

[2] This in turn can lead to a series of developmental consequences ....

[2] In summary, our study shows that ..., where ...

[4] The data presented on these ... clearly show that ...., and we have produced a set of tools with which to investigate further their role in ...

[3] The results of this study have clearly demonstrated that ...

[5] The results of these studies could lead to the development of .., to be used ...

[6] These ... are useful for various applications, such as ....

[6] If the implementation of the techniques presented in this paper is achieved through the use of ...., the ... can be scaled up by ...., offering the potential to produce ...

[7] In addition, we show that ....(technology) can be used to select...

[8] Moreover, the ..... can serve as a model ...to study ...

[9] Our simple and versatile technique offers a novel route to generate ... for biomedical applications and for ... studies of ....

[10] These results confirm that .... is dictated by ...

F. Acknowledgements

- support

[4] This work was funded by ...

[7] We acknowledge ....

[7] This work was supported ....

[9] This work was supported by ....

[10] This work was supported by ....

- helpful discussions

[5] We are grateful to Dr. ..... for providing the ....., Dr. .... for helpful assistance in ....experiments and Prof. ...for..... sequencing.

[9] We thank .... for helpful discussions.

[10] We gratefully acknowledge helpful discussions with ....

[10] We thank ...

G. Caption

FIGURE

[1] Fig. 1. .... expression. Shown are representative .... that were scored with weak (A), moderate (B), and strong (C) ..... Note that ...

[1] Fig. 2. ....expression in .... The frequency of .... expression in .... is shown. Examples of .... are shown.

[1] Fig. 3. .... analyses of .... (A) .... (B) in... (thick line) and .... (hatched line).

[5] Fig. 1. [A] Overview of the .... construct. Expression of .... driven by ...; . The ... gene confers resistance to ....; pUC Ori: origin of replication for the high-copy-number plasmid in E. coli. The other abbreviations refer to ....; bp: base pair. [B] Deduced amino acid sequence of the recombinant ....

[5] Fig. 2. Agarose electrophoretic gel of transformed *P. pastoris* amplicons. Lane M: 1-kb DNA ladder marker; Lane 1: ...; Lane 2: ....'; Lane 3: ...; Lane 4: .. Arrow a: ... gene; arrow b: ...

[5] Fig. 3. SDS-PAGE under reducing conditions of P. pastoris culture media [A] and ...purification steps [B]. [A] Lane 1: ...; Lane 2: ... induced with X% of methanol. [B] Lane 1: ...; Lane 2: ... unbound fraction; Lane 3: .... eluted fraction; Lane 4: ... eluted fraction.

[5] Fig. 4. Effect of ....

III. Example phrases

A. Abstract

A. *Context*

- declare topic's prominence

[2] Folic acid (FA) has traditionally been associated with prevention of neural tube defects; more recent work suggests that it may also be involved in the prevention of adult onset diseases.

#[5] A truncated form of α' chain [tα'], the soybean 7S globulin subunit previously demonstrated to be active in controlling the cholesterol and triglyceride homeostasis in in vitro and in vivo models, was cloned and expressed in the yeast Pichia pastoris.

[7] Affinity reagents, such as antibodies, are needed to study protein expression patterns, sub-cellular localization, and post-translational modifications in complex mixtures and tissues.

[8] Capillary microfluidic devices are used to prepare monodisperse polymersomes consisting of a hydrogel core and a bilayer membrane of amphiphilic diblock-copolymers.

- acquaint terms, processes

[1] Folate receptor α [FRα] is a folate-binding protein overexpressed on ovarian and several other epithelial malignancies that can be used as a target for imaging and therapeutic strategies.

[1] Folate receptor α [FRα] is a folate-binding protein... that can be used as a target for...

- bringing on a technique

[6] Stimuli-responsive polymer microgels can be produced with exquisite control using droplet microfluidics;

[7] Phage Emulsion, Secretion, and Capture [ESCape] is a novel micro-emulsion technology that utilizes water-in-oil [W/O] emulsions for the identification and isolation of cells secreting phage particles that display desirable antibodies.

[7] Rather than using biopanning on a large mixed population, phage micro-emulsion technology allows us to individually query clonal populations of amplified phage against the antigen.

- literature

[3] In a previous study with inhibitors of N-glycosylation, it was proposed that core glycosylation of the folate receptor [FR] is required for the proper folding of the protein.

- exposing an interesting feature of the topic/technique

[2] ... more recent work suggests that it may also be involved in the prevention of adult onset diseases.

- expose hypothesis

[7] Using this method, a large library of antibody-displaying phage will bind to beads in individual compartments.

B. ***Gap***

- quote problems in the area

[1] The goal of this study is to improve historical data that lack specific information about FRα expression in rare histological subtypes, primary disease versus metastatic foci, and recurrent disease.

*[6] however, in existing methods, the droplet templating is strongly coupled to the material synthesis, because droplet solidifcation usually occurs through rapid polymerization immediately after the microfluidic droplet formation.

*[6] This circumstance limits independent control of the material properties and the morphology of the resultant microgel particles.

- quote needings

[2] As the role of FA in human health and disease expands, it also becomes more critical to understand the mechanisms behind FA action.

- quote gap/lack of previous research

[3] In a previous study with inhibitors of N-glycosylation, it was proposed that core glycosylation of the folate receptor [FR] is required for the proper folding of the protein.

[3]The human FR isoforms type a and type b have three and two candidate sites for N-glycosylation respectively, only one of which is conserved.

*[6] however, in existing methods, the droplet templating is strongly coupled to the material synthesis, because droplet solidifcation usually occurs through rapid polymerization immediately after the microfluidic droplet formation.

*[6] This circumstance limits independent control of the material properties and the morphology of the resultant microgel particles.

- quote gap with hypothesis

[7] The use of emulsions to generate microdroplets has the promise of accelerating phage selection experiments by permitting fine discrimination of kinetic parameters for binding to targets.

C. ***Purpose***

- quote the main purpose

[2] In this work we examined the hypothesis that folate receptor alpha [FRa] acts as a transcription factor.

[6] To overcome this limitation, we produce sensitive polymer microgels from prefabricated precursor polymers.

[7] In this study, we demonstrate the ability of phage microemulsion technology to distinguish two scFvs with a 300-fold difference in binding affinities [100 nM and 300 pM, respectively].

[8] Capillary microfluidic devices are used to prepare monodisperse polymersomes consisting of a hydrogel core and a bilayer membrane of amphiphilic diblock-copolymers.

[9] We present a novel approach for fabricating monodisperse phospholipid vesicles with high encapsulation efficiency using controlled double emulsions as templates.

[10] We report the spontaneous formation of emulsion droplets and multilamellar concentric onions when a water drop is immersed into dodecane containing phospholipids.

- detail purpose

[7] In addition, we describe the application of phage microemulsion technology for the selection of scFvs that are resistant to elevated temperatures.

- introduce purpose with gap

[1] The goal of this study is to improve historical data that lack specific information about FRα expression in rare histological subtypes, primary disease versus metastatic foci, and recurrent disease

- introduce purpose with materials and methods

[3] The significance of N-glycosylation at each of these loci in the expression and function of FR was examined by eliminating the sites both individually and in combination by introducing Asn => Gln substitutions.

- introduce purpose with results

[4] A search of the chicken expressed sequence tag [EST] database identified 2 cDNA clones that appeared to represent members of the SULT1B and SULT1C enzyme families.

D. *Materials and Methods*

- list the criteria and conditions

[4] We cloned these cDNAs into the bacterial expression vectors from the pET series.

[4] Transformed Escherichia coli cells strongly expressed the recombinant proteins.

[4] Purification of the recombinant enzymes from E. coli was accomplished by a three-step procedure involving ammonium sulfate precipitation, anion exchange chromatography, and affinity chromatography.

[5] The recombinant polypeptide spanned 216 amino acid residues from the N-terminal side and included the N-terminal extension region of the soybean subunit.

[5] The tα' polypeptide was purified by conventional biochemical techniques, and its potential to modulate the activity of low-density lipoprotein [LDL] receptor was evaluated in a human hepatoma cell line [Hep G2] by monitoring the uptake and degradation of labeled LDL.

- quote/describe materials and methods

[1] FRα expression was analyzed by immunohistochemistry on 186 primary and 27 recurrent ovarian tumors, including 24 pairs of samples obtained from the same individuals at diagnosis and at secondary debulking surgery.

[1] For 20 of the 186 primaries, simultaneous metastatic foci were also analyzed. FRα staining was analyzed in light of disease morphology, stage, grade, debulking status, and time from diagnosis to recurrence and death.

[4] These cDNAs were fully sequenced and found to contain full-length inserts.

*[6] We use microfluidic devices to emulsify semidilute solutions of crosslinkable poly[N-isopropylacrylamide] and solidify the drops via polymer-analogous gelation.

[8] To make polymersomes, water-in-oil-in-water double-emulsion drops are prepared as templates through single-step emulsification in a capillary microfluidic device.

- justify the choice of materials and methods

[6] We use microfluidic devices to emulsify semidilute solutions of crosslinkable poly[N-isopropylacrylamide] and solidify the drops via polymer-analogous gelation.

[8] The amphiphile-laden middle oil phase of the double-emulsion drop dewets from the surface of the innermost water drop, which contains hydrogel prepolymers; this dewetting leads to the formation of a bilayer membrane.

?[8] Subsequently, the oil phase completely separates from the innermost water drop, leaving a polymersome. Upon UV illumination of the polymersome, the prepolymers encapsulated within the interior are crosslinked, forming a hydrogel core.

[9] Glass-capillary microfluidics is used to generate monodisperse double emulsion templates.

??[10] We use coherent anti-Stokes Raman scattering microscopy to determine that the shell of the onion structures is composed of partially hydrated concentric bilayers, and the core is composed of lipids, water, and dodecane.

E. ***Results***

- statement of results /Describe/Discuss

[1] FRα expression was apparent in 134 of 186 [72%] primary and 22 of 27 [81.5%] recurrent ovarian tumors.

[1] FRα status was not associated with time to recurrence or overall survival in either univariate or multivariable analyses.

[2] The work described here shows that FRa translocates to the nucleus, where it binds to cis-regulatory elements at promoter regions of Fgfr4 and Hes1, and regulates their expression.

[3] In both the proteins, the inclusion of each additional N-glycosylation site partly contributed to restoration of cell surface [3H]folic acid binding and receptor-mediated folate transport.

[3] Further, in FR-b the introduction of an additional unnatural site of N-glycosylation resulted in the enhancement of the expression of the cell surface receptor compared with the wild-type protein.

[4] The purified enzymes displayed subunit molecular weights of approximately 35,000Da on SDS–PAGE, as predicted, and were both able to sulfate a wide range of compounds, including xenobiotics and endogenous substrates such as iodothyronines.

[4] Detailed kinetic analysis showed SULT1C1 was more prolific in that it was able to sulfate

dopamine, tyramine, and apomorphine, which SULT1B1 was not.

[4] We also raised antibodies against these proteins, which were able to detect the SULTs by ELISA, and which were able to strongly inhibit the recombinant enzymes.

[5] The LDL uptake [+192%] and degradation [+143%] by cells tested at the highest tα' dose [8 μM] were similar to those found in cells incubated with 1 μM simvastatin, a potent inhibitor of cholesterol biosynthesis. The cell response to tα' was found to be dose dependent.

[5] The use of a recombinant polypeptide ruled out the involvement of any other soybean component.

[6] This approach separates the polymer synthesis from the particle gelation and allows each to be controlled independently, thus enabling us to form monodisperse, thermo- responsive microgel particles with well-controlled composition and functionality.

[8] The hydrogel network within the polymersomes facilitates sustained release of the encapsulated materials and increases the stability of the polymersomes through the formation of a scaffold to support the bilayer.

[9] We show that the high uniformity in size and shape of the templates are maintained in the final phospholipid vesicles after a solvent removal step.

[10] We show that the origin of the spontaneous emulsification is the formation of a semicrystalline multilamellar film at the dodecane-water interface, which swells with water, shedding the emulsion and onion droplets.

F. *Conclusion*

- *exhibit conclusions*

[1] FRα expression occurs frequently, especially in the common high-grade, high-stage serous tumors that are most likely to recur.

[3] The results indicate that the total mass of N-glycosylation, not a specific locus of the modification, is critical for the effcient folding and optimal expression of functional FR-a and FR-b.

- *statement contributions of the work*

[1] New findings from this study show that FRα expression is maintained on metastatic foci and recurrent tumors, suggesting that novel folate-targeted therapies may hold promise for the majority of women with either newly diagnosed or recurrent ovarian cancer.

[2] Until this time FRa has only been considered as a folate transporter, these studies describe a novel role for FRa as a transcription factor.

[4] This is the first detailed characterization of sulfotransferases from the chicken, and it demonstrates that the avian and mammalian SULT1 enzymes are closely related in both structure and function.

[5] These findings open new prospects in the studies aimed at identifying soybean regulatory [poly]peptide[s] and the mechanism involved in this biological response, as a gateway to their utilization for the management of human health.

[6] In addition, the microfluidic templating allows us to form complex particle morphologies such as hollow gel shells, anisotropic microgels, or multi-layered microgel capsules.

[8] In addition, this approach provides a facile method to make monodisperse hydrogel particles directly dispersed in water.

[9] Our simple and versatile technique is applicable to a wide range of phospholipids.

- statement of recommendations to future work

[1] ... suggesting that novel folate- targeted therapies may hold promise for the majority of women with either newly diagnosed or recurrent ovarian cancer.

B. Introduction

A. *Context*

- declare topic's prominence

[1] The folate receptor alpha (FRα) is a glycosylphosphatidyl- inositol-linked protein that is overexpressed in several epithelial malignancies, including ovarian, renal, lung, and breast cancers. (also, introducing terms)

[2] Traditionally folic acid (FA) has been associated with prevention of neural tube defects; however more recently FA has been associated with the prevention of adult onset disease, such as Alzheimer's disease, dementia, neuropsychiatric disorders, cardiovascular diseases, and cerebral ischemia.

[3] The mammalian folate receptor (FR) is a single polypeptide that binds folic acid with a relatively high affinity (Kd < 1 nM) and with a stoichiometry of 1 : 1.

[3] The receptor also binds the major circulating folate coenzyme, [6S]-5-methyltetrahydrofolate and various anti-folate drugs.

[5] The role of dietary soybean proteins in the control of lipidemic levels of hypercholesterolemic

patients is a widely accepted issue.

[7] Affinity reagents, such as antibodies, are commonly used to study protein expression patterns, sub-cellular localization, and post-translational modifications in complex mixtures and tissues.

[9] Vesicles, also known as liposomes, are phospholipid bilayer membranes which surround aqueous compartments.

[10] In some instances, however, the initial composition of the system can be chosen so that emulsion droplets or onions can form spontaneously.

- introduce terms, processes obs: vem bastante com literatura

[1] While the function of FRα in tumors is unclear, in the kidney it serves as a high-affinity salvage receptor that retrieves folate from the filtrate and returns it via transcytosis to the blood; in the brain, it likely concentrates folate in cerebrospinal fluid.

[1] Unlike the more ubiquitously expressed reduced folate carrier and proton-coupled folate transporter that regulate folate homeostasis, FRα allows internalization of folic acid that has been conjugated to low molecular weight compounds, proteins, or nanoparticles.

[4] Sulfation is a ubiquitous process in nature that performs many functions in homeostasis, body structure, and chemical defence.

[6] Stimuli-responsive or "smart" microgels are micrometer-sized polymer particles that are able to swell or shrink in response to changes in their surrounding.

[8] Polymersomes, vesicle structures whose membrane is composed of a bilayer of amphiphilic block-copolymers, have great potential for encapsulation and controlled delivery of active materials due to their high stability and low permeability by comparison with liposomes composed of a lipid bilayer.

[10] The production of emulsion droplets and of concentric multilamellar structures called onions usually requires the addition of energy to the system.1-3

B. ***Literature review***

- historical review of literature/background

[1] For example, MORAb-003, a humanized, high-affinity monoclonal antibody against FRα based on the murine LK26 clone, is currently undergoing phase II testing in ovarian cancer patients after showing cell-mediated cytotoxicity, complement-dependent killing, and non-immune mediated, FRα-dependent inhibition of growth under folate- limiting conditions.

[3] Multiple isoforms of FR have been identified from human (hFR-a, hFR-b and hFR-c) and murine (mFR-a and mFR-b) sources, and they share amino acid sequence identity of 68–79%

including 16 cysteine residues that are all conserved.

[5] In previous studies , the direct involvement of one subunit of the soybean 7S globulin, the α' subunit, in the up-regulation of the low- density lipoprotein (LDL) receptor (LDL-R) was demonstrated in in vitro and in vivo systems, suggesting that biologically active [poly] peptides, capable of modulating cholesterol homeostasis, are likely to be produced by cell enzyme processing.

[5] On the other hand, since the molecular weight of the α' subunit is around 71 kDa, it seems unlikely that it may cross in vivo the intestinal barrier with no modification.

[9] This approach has been applied to form polymersomes, vesicular structures composed of a bilayer of amphiphilic diblock copolymers.

[10] Four possible mechanisms have been proposed to explain the spontaneous formation of emulsion droplets. Diffusion of water into the oil can result in nucleation and swelling of water-surfactant aggregates.

[10] Alternatively, spontaneous emulsification may be caused by a reduction of the surface tension, although this mechanism is still debated.

[10] Finally, spontaneous emulsification may be caused by the self- assembly at the interface of surfactant into an ordered multilayer film similar to a lyotropic liquid-crystalline phase, which can swell by diffusion of water or oil and form droplets which are then shed from the interface.

- current trends

[1] This property has implications for targeting of chemotherapeutic drugs, cytotoxic viruses, or imaging agents to FRα-expressing cells.

[1] FRα is an attractive candidate for targeted biologic therapy of ovarian cancer.

[6] A very common material with pronounced responsiveness is poly[N-isopropylacrylamide] [pNIPAAm]: it exhibits a lower critical solution behavior in aqueous media at temperatures around 32 $_{-}^{C}$ and has thus been used extensively to form thermo-responsive microgels.

[6] Since the transition temperature of pNIPAAm shifts upon copolymerization with polar or non-polar comonomers, the sensitivity of pNIPAAm microgels can be controlled by their chemical composition.

[7] Rather than using biopanning on a large mixed population, this method allows us to individually query clonal populations of amplified phage against the antigen. Depending on droplet size, a 100 ll reaction mix dispersed in 0.5 ml oil can form 2 □ 1010 aqueous compartments suitable for cell growth.

[7] The use of emulsions to generate microdroplets has the promise of accelerating phage

selection experiments by permitting fine discrimination of kinetic parameters for binding to targets.

[8] In addition, polymersomes with multicompartments are prepared by a microfluidic emulsification, which enables the encapsulation of multicomponent ingredients while avoiding cross-contamination.

[8] Recent advances in microfluidics have enabled the production of such polymersomes with uniform size and high encapsulation efficiency, which is difficult to achieve with conventional approaches such as electroformation or bulk hydration of dried amphiphiles.

[9] A new strategy to form phospholipid vesicles with high uniformity in size and high encapsulation efficiency is to utilize templates to generate the vesicles.

[9] An example is the use of simple water-in-oil (W/O) emulsions as templates.

[9] Monodisperse emulsion templates can be generated using microfluidics.

[10] This is of great interest for potential applications such as the delivery of agricultural chemicals and drugs and for oil recovery processes and thus has stimulated extensive studies of the phenomenon.

- progress in the area

[2] This study examines a possible role of another caveolar protein, FRa as a transcription factor for key developmental genes.

[4] Recently, reports of characterization of SULTs from zebrafish have appeared.

[4] One of these is clearly related to sulfotransferases of family 6 from chicken [Gallus gallus] [10] and Xenopus laevis [EMBL Database Accession No. BC041544], whilst the others do not fall clearly into any of the known SULT families, although they may be most closely associated with the phenol sulfotransferase family, SULT1.

[4] It has also been shown that sulfation of iodothyronines is carried out by cytosolic fractions of chicken liver, kidney, and brain.

[7] Previously, production of an antibody to a particular protein required immunization of animals [i.e., mice, rabbits] and recovery of antibodies from their sera.

[7] This is in contrast to newer methods that involve the generation of large libraries of recombinant antibodies and the in vitro screening of these libraries for the desired binding properties.

[7] Through the use of appropriate counter-selection steps, it is possible to guide recognition of new epitopes or discriminate between certain molecular features of a target (i.e., conformation,

post-translational modification).

[7] Thus, there has been a great deal of recent interest in tapping the potential of recombinant affinity reagents for characterizing the human proteome, and the early comparisons to traditional antibodies are promising.

[7] Phage display is the most widely used method for selecting binding molecules from recombinant antibody libraries.

[8] The inner compartment of polymersomes can be used to encapsulate large amounts of water-soluble ingredients, and these encapsulants can be released upon triggered breakup of the bilayer membrane.[

[9] Due to the random nature of the bilayer folding, these methods typically lead to the formation of vesicles that are nonuniform in both size and shape.

[9] Moreover, the encapsulation efficiency of these processes is quite low, generally less than 35%.

[9] Since phospholipids are an integral component of biological membranes, phospholipid vesicles also provide ideal platforms for the study of the physical properties of biomembranes.

[10] Spontaneous emulsification of direct [water-in-oil] emulsions has been reported for two surfactant systems: AOT [Aerosol OT, sodium bis[2-ethylhexyl] sulfosucci- nate],7 an anionic surfactant also known to stabilize microemulsions, and C12E6 [dodecylhexaglycol polyoxy-ethylene 6 lauryl ether],8 a nonionic surfactant that can promote spontaneous emulsification when a cosolvent soluble in both oil and water is present.

- state of art

[1] The tumor specificity and high levels of FRα expression and the potential to boost immunity to tumors with FRα-specific approaches have generated significant enthusiasm for testing strategies targeting FRα in ovarian cancer patients.

[6] It is this responsiveness which makes them attractive for applications in various fields such as drug delivery, catalysis, sensing, and photonics.

[6] A powerful method to form pre-microgel droplets with exquisite control is droplet microfluidics.

[9] They are promising delivery vehicles for drugs, enzymes, and gases, and bioreactors for biomedical applications.

- previous author's work

[2] Previous data from our lab demonstrated that FA remodels chromatin structures.

[5] From these statements, our strategy was to test the effectiveness of both polypeptides with a hydrolyzed isoflavone-free soybean concentrate and synthetic peptides having amino acid sequences that differed between the 7S soybean globulin subunits on the LDL-R modulation by Hep G2 cells.

[7] We have previously described a novel approach that utilizes water-in-oil (W/O) emulsions for the identification and isolation of cells secreting phage particles that display desirable scFvs.

[7] The water-in-oil emulsion has been applied to the in vitro compartmentalization of biochemical reactions, including selecting catalysts of DNA methyltransferases and for the directed evolution of Taq DNA polymerase.

[7] Using the method we developed , a large library of antibody-displaying phage will bind to beads in individual compartments (Fig. 1).

C. *Gap*

- unsolved conflicts

[1] What is unknown in attempting to apply novel FRa-based therapeutics is the FRα-expression status of tumors with more rare histologies, the stability of expression of FRα across the multiple sites of disease typically present at diagnosis in ovarian cancer and whether FRα expression is lost or maintained with disease recurrence.

[2] A second mechanism of FA action may be through FRa translocating to the nucleus and acting as a transcription factor.

[3] It has been demonstrated that deglycosylation of FR-a from KB cells with endoglycosidase H or of a mixture of FR-a and FR-b purified from placenta with N-glycanase does not affect the ability of the proteins to bind folate.

[3] In contrast, in a systematic study with inhibitors of glycosylation, it was demonstrated that core glycosylation is essential for the acquisition of the ligand-binding property of FR-a in KB cells.

[6] However, the microgel behavior also depends on geometric parameters such as the particle size and shape; it is therefore crucial to control both the particle morphology and polymer functionality to design advanced micro-materials with optimized performance.

- restriction of previous work

[1] In addition, although one investigative team has stated that FRα overexpression is an indicator of platinum resistance in ovarian cancer, the study was small and performed in the pre-taxane era.

[2] Bozard and colleagues reported the presence of FRa on the plasma membrane, the nuclear membrane and within endosomal structures; however the relevance of nuclear FRa was not determined.

[5] There is a paucity of information on non-mammalian sulfotransferases, however.

[5] Plants have a number of different sulfotransferases [the Arabidopsis thaliana genome SULT-like genes] that are related to the mammalian SULTs and are involved in metabolism of flavonols, brassinosteroids, and other signalling molecules but, to date, there is little information on SULTs in non- mammalian animal species.

[8] However, despite this progress, the use of such polymersomes is limited due to the poor stability of the molecular bilayer under osmotic pressure or mechanical stress, which leads undesired rupture of the membrane.

[8] Moreover, the encapsulated materials dissolved in the aqueous core are quickly released upon destruction of the membrane, making it difficult to use the polymersomes for controlled release of ingredients.

[10] When decane containing lecithin is placed in contact with water, a macroscopic semicrystalline film can develop at the interface and interfacial waves can form, with wavelengths of the order of millimeters; however, it is unclear whether spontaneous formation of emulsion droplets or onions occurs.

- issues not yet considered

[9] Another strategy is to apply pulsed microfluidic jets to deform planar lipid membranes into vesicle-like compartments and vesicles.

[9] However, it is difficult to precisely control such processes for vesicle formation.

[10] By contrast, to our knowledge, no observations of spontaneously formed inverted (oil-in-water) emulsions have been reported.

[10] Thus, spontaneous emulsification with lipids is still an unexplored phenomenon.

D. ***Purpose***

- State a new approach or methodology or technique

[4] Since sulfation, catalyzed by a number of SULT isoforms of the family 1, is an important pathway of iodothyronine metabolism and deactivation in humans, we were interested to discover whether the chicken also possesses SULTs with the capacity to sulfate iodothyronines.

[6] One idea to control the size and shape of microparticles is to use emulsion droplets as templates for the particle synthesis; the key element of this strategy is to control the morphology of the pre microgel droplets and to retain their shape by subsequent droplet solidification.

[8] In this paper, we report a microfluidic approach to make polymersomes composed of a hydrogel core and a bilayer membrane of biocompatible amphiphilic diblock-copolymer; the hydrogel core provides enhanced stability of the bilayer and sustained release of the encapsulants.

[9] Here, we present a novel technique for forming phospholipid vesicles using monodisperse double emulsions with a core-shell structure as templates.

- Explain purpose

[3] The present study was undertaken to identify the candidate sites of N-glycosylation in hFR-a and hFR-b at which the modification can occur *in* vivo and to test the role of specific sites of N-glycosylation in the expression of functional FR.

[3] Our working hypothesis was that the single candidate site of N- glycosylation that is conserved among the five human and murine FR polypeptides is either the most significant or the only contributor to the formation of a functional receptor.

[9] Because of the resemblance of core-shell structures to vesicular structures, techniques that rely on double emulsion templates should be robust and straightforward.

- Present improvements / advances of a topic in the literature

[2] In this work we tested the hypothesis that in response to FA, FRa translocates to the nucleus and acts as a transcription factor.

- Present an extension of a previous work of the author

[5] For this reason, our research has been directed to seeking the amino acid sequence(s) of α' subunit responsible for the pharmacological effect.

[5] On the other hand, since the study obtained with small peptides is still currently under investigation and has not been conclusive so far, we decided to develop a different strategy, aimed at identifying an active limited region of the α' chain.

[7] In this study, we demonstrate the application of phage micro-emulsion technology for directed evolution experiments.

- Specify the purpose

[1] We performed this study to define the extent of FRα expression in ovarian cancers of different histologies, grades, and stages and to determine whether FRα status was associated with time to

recurrence and overall survival.

[6] In this paper, we review our work on the use of microfluidic devices to produce thermo-responsive pNIPAAm microgels from macromolecular precursors.

[10] In this paper, we study the interfacial phenomena occurring at a dodecane-water interface when lipids are initially dispersed in dodecane.

- Introduce more purposes

[1] In addition, we examined multiple metastatic sites from a subset of patients with advanced disease at diagnosis as well as matched samples from patients at diagnosis and subsequent recurrence.

E. *Materials and Methods*

- list the criteria and conditions

[8] Using a capillary microfluidic device, we prepare monodisperse water-in-oil-in- water [W/O/W] double-emulsion drops which are transformed into polymersomes through a dewetting of amphiphileladen middle oil phase onto the surface of innermost drop containing hydrogel prepolymers.

- quote/describe materials and methods

- justify the choice of materials and methods

[3] To address these issues, we used a mutagenesis approach to either eliminate potential glycosylation sites in hFR-a and hFR-b systematically or introduce an unnatural glycosylation site in hFR-b.

[3] We studied the effects of these changes on the glycosylation of the FR polypeptides, their ability to bind [3H]folic acid and the extent of their expression on the cell surface as functional folate- binding and transport proteins.

[5] To this purpose, the heterologous expression of a truncated form of the α' chain, which will be referred to as tα' in this article, was undertaken.

[9] In our approach, phospholipids are dissolved in a mixture of volatile organic solvents that is immiscible with aqueous phases.

F. *Results*

- statement of results

[5] The objective was achieved in secretion-competent cells of the yeast Pichia pastoris.

[5] The recombinant polypeptide was purified and its biological activity assessed in HepG2 cells.

[5] These data suggested a potentially promising area for the development of dietary peptides characterized by important pharmacological properties.

[8] Upon UV-irradiation, the prepolymers encapsulated in the polymersomes are crosslinked, resulting in hydrogel cores covered by a bilayer membrane.

- remark/discuss results and possible problems

[4] This represented the first member of a new SULT family (now provisionally named SULT6), of no known endogenous or chemical defence function.

[4] The recombinant (CHICK)SULT6A1 was able to sulfate corticosterone and b-estradiol, but no more information about this enzyme is available.

[5] The results of these studies pointed out that a marked LDL-R up- regulation could be induced in HepG2 cells exposed to partially hydrolyzed polypeptides ranging from 3000 to 20,000 Da, as well as to a small 7S soybean synthetic peptide (2271 Da) at a concentration of 10!4 M (9).

[8] The hydrogel network facilitates the sustained release of encapsulated materials owing to a considerable increase of the diffusion path and a decrease of diffusivity.

[8] In addition, the swollen hydrogel supports the bilayer membrane, preventing its sharp deformation when subjected to external stress; this leads to enhanced stability of the polymersomes.

[9] Such phospholipid-stabilized W/O/W double emulsion drops have previously been used as vesicle-like compartments for encapsulation; however, in our work, we use them as templates to direct the formation of phospholipid vesicles by removing the solvent in oil phase through evaporation, as illustrated in Scheme 1.

[9] However, phospholipid bilayers are too fragile to undergo the solvent removal step without breakage; thus, this method has not been successfully applied to phospholipid vesicles.

G. *Conclusion*

- exhibit conclusions

[4] As a first step towards determining the suitability of the chicken as a model system for the

study of iodothyronine sulfation, we report here the cloning, sequence analysis, expression, purification, and characterization of two members of the chicken SULT1 family belonging to the SULT1B and SULT1C subfamilies.

[5] By the use of this biotechnological approach, adequate amounts of the recombinant polypeptide could be obtained and tested in in vitro trials, as a first step to further in vivo assays

[8] Moreover, we demonstrate that these polymersomes provide facile templates for the production of monodisperse hydrogel particles directly dispersed in water.

[9] Here, we introduce new strategies to improve the stability of phospholipid vesicles during solvent removal.

- statement contributions/value of the work/research

[9] Our technique can be used to create phospholipid vesicles with different composition while maintaining high size uniformity and encapsulation efficiency.

- statement of recommendations to future work

C. Materials and Methods

A. Materials

- list, source, informations

[1] Ovarian cancer specimens from women who had surgery at Mayo Clinic Rochester between June 15, 1991 and June 15, 2005 were eligible for inclusion in the study.

[3] All of the mutants were constructed by using PCR.

[4] Protein purification columns and media were purchased from Amersham–Pharmacia Biotech (Little Chalfont, UK).

[5] *Pichia pastoris X33* (wild-type) strain (Invitrogen, San Diego, CA, USA) was used for yeast expression.

[5] The bacterial strain utilized for genetic manipulations was E. coli XL1- Blue (Invitrogen).

[5] Restriction enzymes PstI and XbaI were purchased from Roche (Indianapolis, IN, USA) and SacI from Fermentas (Ontario, Canada).

[5] Taq DNA polymerase was purchased from Invitrogen.

[5] Zeocin was purchased from Invivogen (San Diego, CA, USA). ..provided, we bought ...

[5] The oligonucleotides for PCR were obtained from Primm (Milan, Italy).

[5] Peptone, tryptone, yeast extract and agar were purchased from Becton Dickinson (Sparks, MD, USA).

[5] Other chemicals were reagent grade from Sigma (St. Louis, MO, USA).

[7] The synthetic anti-MS2 single-chain variable fragment (scFv), AFX704, was generated by CDR-grafting of an antibody provided by James Carney at Aberdeen Proving Ground and amplified by error prone PCR.

[7] The PCR reaction contained 7 mM MgCl2, 50 mM KCl, 10 mM Tris (pH 8.3), 0.01% gelatin, 0.2 mM dGTP, 0.2 mM dATP, 1 mM dCTP, 1 mM dTTP, 0.5 mM MnCl2, 30 pmol of each primer, 20 femtomol of input DNA, and 5 units of Taq polymerase.

[8] To make hydrogel core in polymersomes, we use 10 wt% or 15 wt% aqueous solution of PEGDA (Mw 4000 g/mol, Polyscience, Inc.) containing 0.2 wt% 4-(2-hydroxyethoxy)phenyl- (2-hydroxy-2-propyl)ketone as a photoininitator.

[9] The inner phase of the water-in-oil-in-water (W/O/ W) double emulsion droplets was made of 0-5 wt % poly(vinyl alcohol) (PV A; *Mw:* 13 000-23 000 g · mol-1, 87-89% hydrolyzed, Sigma-Aldrich Co.) and ~0.02 wt % 1 μm yellow-green sulfate- modified microspheres (Fluosphere, Invitrogen, Inc.).

[9] Unless otherwise noted, the middle organic phase consisted of 5-10 mg · mL-1 lipids with 0.02 mol % Texas red labeled 1,2-dihexanoyl-sn-glycero- 3-phosphoethanolamine (TR-DHPE) for fluorescent visualization in an organic solvent mixture of toluene (EMD Chemicals, Inc.) and chloroform (Mallinckrodt Chemicals, Inc.) in 1.8:1 volume ratio.

[9] Experiments were conducted with the following lipids: 1,2- dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl- *sn-glycero-3-phosphocholine* (DMPC), 1,2-dioleoyl-sn-glycero-3- phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3- phoscholine (POPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diacyl-sn-glycero-3-phospho-L-serine (DPPS), and Texas red labeled 1,2-dihexanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE).

[9] All lipids were purchased in powder form from Avanti Polar Lipids, Inc.

[9] The outer phase was either a 10 wt % PV A solution or a 40 vol % glycerol and 2 wt % PVA solution.

[9] Solutions and solvents were all filtered before introduction into glass microcapillary devices.

[9] Water with a resistivity of18.2 MΩ · cm-1 was acquired from a Millipore Milli-Q system.

[10] The aqueous solution used in these experiments was a 5 mM Tris buffer containing 100 mM sodium chloride at pH] 7.4. Anhydrous dodecane (Sigma, Saint Louis, MO) was used to prepare two different dodecane solutions.

[10] A dodecane solution saturated with water was prepared by stirring 100 mL of buffer into 1 L of dodecane for 24 h; subsequently excess buffer was removed from the dodecane.

[10] Dry dodecane solution was prepared by adding 1 vol % of heavier silicone oil to the anhydrous dodecane; the addition of silicone prevents the diffusion of water into dodecane.

[10] Finally, fully deuterated dodecane (Cambridge Isotope Laboratories; Cambridge, MA) was used for the CARS experiments. The phospholipids (Avanti Polar Lipids, Alabaster, AL) POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and POPS (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine) were used, without further purification, within 2 months of purchase.

[10] These lipids were stored in chloroform and kept in a freezer at -20 °C to prevent degradation.

[10] To prepare the dodecane-lipid suspension used for these experiments, we first formed a dry lipid film of known mass by evaporating the chloroform from the stock solution under nitrogen.

[10] After the film was formed, dodecane was added to resuspend the lipids at the desired concentration, and the dispersion of the film was achieved by sonicating the dodecane- lipid suspension for 30 min.

[10] Finally, the solution was left to stabilize overnight at 30 °C.

B. Methods

- specific information, justifications

[1] Invasive tumors encompassing serous, endometrioid, clear cell, mucinous, or mixed morphologies were selected prior to retrieval of any clinical information.

[1] The specimens chosen for the study were similar to all available patients in this time period in terms of patient age, stage of disease, and grade.

[1] There was a difference between the sample set and all available samples in the distribution of tumor morphologies.

[1] This was caused by oversampling the rare morphologies, which resulted in !56% of the primary tumors studied (compared to 70% of all eligible epithelial malignancies) having serous morphology.

[1] Hematoxylin and eosin-stained slides from the initial surgery were reviewed by a gynecologic

pathologist (GLK) to confirm disease morphology and grade.

[1] Samples were examined for FRα expression by assessing triplicate 0.6 mm cores of formalin-fixed, paraffin-embedded tumors in tissue microarrays (TMAs) or slides made from a full paraffin block.

[2] To test the role of of FRa as a transcription factor we examined nuclear localization in cell lines and interaction of FRa with two candidate genes FGFR4 and Hes1.

[2] These candidate genes were chosen because in our previous studies, working with neural stem cells from Pax3 mutant (also known as or Splotch ($Sp^{-/-}$)) mouse embryos, we found that FA up-regulates Fgfr4 and Fgfr4 receptor protein and increases levels of Hes1.

[3] The resulting PCR products were digested at both ends with the restriction enzymes and substituted for the corresponding fragments in the cDNA species for FR-a and FR-b in the plasmid pCDNAIneo (Invitrogen).

[3] Two complementary oligonucleotides (Table 1), containing the desired mutations, were used in combination with upstream and downstream primers containing appropriate restriction sites.

[3] The recombinant plasmids were amplified in *Escherichia coli* MC1061}p3 and the entire cDNA sequence was verified by dideoxy sequencing with Sequenase Version 2.0 (USB).

[3] Human 293 fibroblasts were grown in Eagle's minimal essential medium (Irvine Scientific, Santa Anna, CA, U.S.A.) supplemented with fetal bovine serum (10%, v}v), penicillin (100 i.u.}ml), streptomycin (100 μg}ml) and l-glutamine (2 mM). Transfections were performed with lipofectamine (Gibco BRL, Grand Island, NY, U.S.A.) in accordance with the manufacturer's protocol.

[3] Cells (3¯10') at 48 h after transfection were washed in a 35 mm tissue culture dish at 4 ∞C once with 1 ml of Hanks balanced salt solution (HBSS), twice with 1 ml of acid saline [10 mM sodium acetate (pH 3.5)}150 mM NaCl], and once again with 1 ml of HBSS.

[3] The cells were then incubated with 2 pmol of [$H]folic acid (Moravek) in 1 ml of HBSS at 4 ∞C for 1 h.

[3] After the cells had been washed once with 1 ml of HBSS, [$H]folic acid bound to the cell surface was recovered in 0.5 ml of acid saline and subjected to liquid- scintillation counting.

[3] The specific [3H]folic acid binding was determined by subtracting the value for the negative control cells, which had been transfected with the vector alone.

[3] The specificity of [3H]folic acid binding was also established by competition with unlabelled folic acid (1 μM).

[3] Cells (3¯10') were dissolved in 10 mM sodium phosphate (pH 7.5)}150 mM NaCl}1% (v}v) Triton X-100 and insoluble material was removed by centrifugation at 10000 ***g*** for 2 min.

[3] Simultaneously, Triton X-100 was added to a final concentration of 1%.

[3] The samples were incubated with 2 pmol of [3H]folic acid at 37 ∞C for 2 h; [3H]folic acid-binding protein was assayed by the charcoal binding assay described previously.

[3] Cleavage of the GPI membrane anchor of hFRs-a and hFRs-b and their quantitative release from the cell surface were accomplished by treating the cells with PI-PLC as described previously.

[3] Insoluble material was removed by centrifugation at 10000 ***g*** for 2 min.

[4] The PAP35S barium precipitation assay was used to measure SULT activity with 4-nitrophenol, dopamine, apomorphine, tyramine, 2-bromophenol, isopro- pylcatechol, and 4-nitrocatechol.

[4] Protein, incubation time, and substrate concentrations were optimized for each assay.

[4] Samples were assayed in duplicate with a control incubation containing vehicle in place of substrate.

[4] Reaction mixtures (final volume 160 mL) comprised 10mM potassium phosphate (pH 7.4), PAPS (20 mM final concentration, containing 0.04 lCi PAP35 S), recombinant SULT enzyme and substrate, unless otherwise indicated.

[4] The reactions were incubated for 10 min at 37 °C before being stopped by placing on ice and the addition of 200 ml of 100 mM barium acetate.

[4] This was then followed by the addition of 200ml of 100 mM barium hydroxide and 200 ml of 100 mM zinc sulfate causing the precipitation of unreacted PAPS.

[4] The samples were then centrifuged at 13,000g for 4 min.

[4] Five hundred microliters of the supernatant was then mixed with 4ml scintillation fluid and the radioactivity was quantified by liquid scintillation spectrometry.

[4] For b-estradiol, a-ethinylestradiol, and DHEA, SULT enzyme activity was measured using 3H-labeled substrate and a solvent extraction procedure to separate sulfated product and unreacted steroid.

[4] The assay buffer was 100 mM Tris/Cl□ and 20 mM MgCl2 (pH 6.0).

[4] The reactions were incubated at 37 °C for 20 min before being stopped by placing on ice followed by the addition of 300 ml of ice-cold H2O.

[4] ...activities were measured essentially as described by Reyns et al.

[4] Fractions were collected, and radioactivity in those with sulfated iodothyronine was corrected for radioactivity detected in the corresponding fractions from the control incubations.

[4] For determination of kinetic parameters, pilot incubations with a number of substrate concentrations were carried out to determine a suitable range over which the estimates could be made.

[4] Assays were performed over the range of concentrations, and Vmax and Km values were estimated using the kinetics module of Prism 4.

[4] Following sequence analysis, we designed PCR primers to amplify the predicted coding regions of these cDNAs incorporating restriction enzyme recognition sequences to facilitate cloning into either pET15b (SULT1C1) or pET17b (SULT1B1) for expression in Escherichia coli.

[4] PCR was performed as previously described, using the following oligonucleotide primers: for 1B1, forward primer 5'-AGCC ATATGGGCACAGTGGA-3' incorporates an NdeI site and reverse primer 5' -GAGAAGGATCCTC AGATGTGTG-3' inserts a BamHI site; for 1C1 forward primer 5' -ATATTTATTGACACCATGGCC CTG-3' introduces a NcoI site and reverse primer 5'- CTTCGGATCCTAATAATCACAATTCC-3' incorporates a BamHI site.

[4] Overnight cultures were used to inoculate 500ml Luria broth supplemented with 100 mg/ml ampicillin and 34 mg/ml chloramphenicol.

[4] Expression was induced, once an A595 of 0.6 was reached by the addition of IPTG to give a final concentration of 1 mM.

[4] The cultures were then incubated for approximately 16 h at 30 °C, with shaking at 200rpm.

[4] Cell-free extract (CFX) was produced by centrifuging the cultures at 4000g for 20 min at 4 °C.

[4] The supernatant was then decanted and the pellets were frozen at 20 °C for at least 1 h.

[4] After lysis of the cells, the residue was resuspended in 40 mM Tris/Cl, pH 8.0, 20mM MgSO4 with Brij 58 added to a final concentration of 0.5% v/v, and centrifuged at 27,000g for 40 min at 4 °C.

[4] The resultant supernatant was used to purify the recombinant proteins exactly as described previously, using ammonium sulfate precipitation, anion exchange chromatography, and affinity chromatog- raphy on 30 50 -ADP agarose.

[4] For immunoinhibition experiments, E. coli cell-free extracts (prepared from three individual colonies) were first incubated with a mixture of antiserum and pre- immune serum in varying proportions between 0:10 and 10:0 for 15min at room temperature.

[4] Protein concentrations were estimated by the methods of either Lowry et al. or Bradford, using

bovine serum albumin as standard.

[4] Proteins were resolved on denaturing SDS–PAGE (12.5% acrylamide monomer), and visualized by Coomassie blue staining.

[5] Pichia pastoris X33 strain was cultured in yeast peptone dextrose (YPD) complete medium (2% peptone, 1% yeast extract, 2% glucose).

[5] The Mut+ transformants were selected in plates containing YPD, agar (1.5%), 100 mg/ml zeocin.

[5] All yeast cultures were maintained at 30°C.

[5] For protein production, selected Zeor-Mut+ transformants were cultured to an optical density of 5 at 600 nm in yeast peptone sorbitol (YPS) medium (2% peptone, 1% yeast extract, 2% sorbitol).

[5] Then methanol was added to 1% final concentration.

[5] All bacterial transformants were selected in plates of low-salt Luria-Bertani [LB] medium containing zeocin (1% tryptone, 0.5% yeast extract, 0.5% sodium chloride, 1.5% agar, 25 mg/ml zeocin).

[5] The tα' gene was amplified by PCR on an expression E. coli plasmid DNA template (pT7-7) containing the sequence of interest (tα').

[5] The oligonucleotide PIC-1 (5'-GAAGGAGATATACATGCTGCAGTGGAGGAAG-3') was designed to generate a PstI restriction site at the 5' end of the tα' gene.

[5]A second oligonucleotide PIC-2 (5'-CGATGCTCTAGATCATCAGTTAAGGATAACGATG-3') was designed to generate an XbaI restriction site at the 3' end of the tα' gene.

[5] Both oligonucleotides were dissolved in MilliQ (Waters, Milan, Italy) sterile water.

[5] The PCR mixture consisted of 0.5 mM primers, 0.8 mM dNTPs (Eppendorf, Hamburg, Germany), 30 ng of template [pT7-7/ t!!], 2.5 U Taq DNA polymerase, PCR buffer (final composition: 50 mM KCl, 1.5 mM MgCl2, 20 mM Tris-Cl (pH 8.4) and sterile water to a final volume of 25 mL).

[5] PCR amplification was carried out on a Perkin Elmer Geneamp PCR System 2400 thermocycler (Perkin Elmer, Wellesley, MA, USA) using the following conditions: initial denaturation (start): 97°C for 6 min; 30 cycles at 97°C for 1 min, 50°C for 1.5 min, 72°C for 1.5 min; final extension: 72°C for 10 min and maintained at 4°C.

[5] The PCR 650- bp product was cloned into the pPICZaB vector resulting in the pPICZaB-tα' construct and transformed in XL1-Blue E. coli cells.

[5] Positive clones were selected on semisolid LB media containing tetracycline and zeocin.

[5] One of these clones was sequenced by Primm (Milan, Italy) to ensure that no mutation occurred in the pPICZaB-tα' construct sequence.

[5] Thirty-five micrograms of the pPICZaB-tα construct and 30 μg of the expression vector pPICZaB (negative control) were linearized by digestion with the restriction enzyme SacI and then purified.

[5] Wild-type yeast cells were transformed by electroporation with 35 μg of linearized pPICZaB-tα' construct and 30 μg of linearized pPICZaB on an Eppendorf (Hamburg, Germany) electroporator 2510 apparatus set at 1.5 kV.

[5] Transformants were first selected by plating on YPD plates containing 100 mg/mL of zeocin.

[5] In order to verify the integration of the construct into transformed P. pastoris genome, the Dneasy Plant Mini Kit (QIAGEN, Hilden, Germany) was used to extract genomic DNA.

[5] Genomic DNA was used as template to verify the insertion of the construct at the alcohol oxidase promoter (AOX1) site by PCR using 5'AOX1 [5'-GACTGGTTCCAATTGACAAGC-3'] and 3'AOX1 primers [5'-GCAAATGGCATTCTGACATCC-3'].

[5] PCR was performed using the following conditions: initial denaturation [start]: 94°C for 10 min; 30 cycles at 94°C for 40 s, 60°C for 40 s, 72°C for 20 s; final extension: 72°C for 10 min and maintained at 4°C.

[5] Eighteen Zeo+ transformants and one transformant containing pPICZaB expression vector [negative control] were grown in 50 mL of YPS medium at 30°C in a shaking [180 rpm] incubator to an OD600 of 5.

[5] The inducing phase was triggered by adding methanol to 1% final concentration and prolonged for 24 h.

[5] Aliquots of the supernatants were examined for expression of tα' by SDS-PAGE.

The clone transformant with the highest tα' expression was selected for large-scale production of the recombinant protein and grown under the same conditions as above.

[5] About 1 L of culture was centrifuged at 11,300×g and 24°C for 15 min with a J2-21 M/E centrifuge (Beckman Instrument, Palo Alto, CA, USA).

[5] The proteins contained in the supernatant were precipitated by 70% ammonium sulfate and centrifuged at 11,300×g at 4°C for 30 min.

[5] The pellet was dissolved in 50 mL sterile water and precipitated by 100% acetone (1:1 v/v) at !30°C.

[5] The solution was centrifuged for 1 h as above.

[5] The pellet was dissolved in 25 mL 50 mM Tris-HCl (pH 7.4) and loaded on a DEAE-cellulose column (2.5×10 cm, Whatman, Maidstone, UK) equilibrated with the same buffer.

[5] The elution of retained proteins was carried out with the same buffer containing 0.15 and 0.25 M NaCl, respectively.

[5] The fraction eluted with 0.25 M NaCl displayed the greatest content of tα'.

[5] SDS-PAGE under reducing conditions (2% β-mercaptoethanol) was carried out on 12% polyacrylamide gels using a mini-gel kit (Protean II cell, Bio-Rad).

[5] The gels were stained with Coomassie blue.

[5] In order to assess cell viability, culture media from cells exposed to tα' at different concentrations were tested by MTT assay, essentially as described in Ref. [9].

[5] Cell enzyme leakage was determined by measuring lactate dehydrogenase (LDH) activity, using a kinetic (LDH/LD) diagnostic kit (Sigma Diagnostics).

[5] Differences in cell uptake and degradation of LDL after cell incubation with tα' at different concentrations were determined by ANOVA followed by Dunnett's test. Values are expressed as means±S.D.; P values b.05 were considered as statistically significant.

[6] We fabricate these microgels by microfluidic emulsification of semidilute solutions of crosslinkable pNIPAAm, followed by subsequent droplet gelation through a polymer-analogous reaction, as illustrated in Fig. 1.

[6] Using pre-functionalized precursors allows us to obtain particles with well-defined amounts of functional sites.

[6] Due to the control achieved through the microfluidic templating, these particles are highly monodisperse, and their size is determined.

[6] In addition, this approach allows us to form complex microgel structures such as hollow gel shells, anisotropic microgels, or multi-layered microgel capsules.

[6] To form microgels from macromolecular precursors, we use linear pNIPAAm chains with pendant dimethylmaleimide [DMMI] side groups; these polymers can be crosslinked through a photochemical reaction based on the triplet-sensitized dimerization of their DMMI moieties, as shown in Fig. 2 [35,36].

[6] For this purpose, we emulsify aqueous precursor solutions with concentrations in the semidilute unentangled regime, an intermediate range right above the threshold for coil overlap, c*, yet below the onset of chain entanglement, ce*.

[7] #The scFvs specific for the MS2 coat protein were generated by phage display affinity maturation of an antibody provided by James Carney at Aberdeen Proving Ground.

[7]#Cells expressing phage displaying the scFvs were incubated with MS2-coated magnetic beads [5 ll; 7 □ 108 beads/ml] and emulsified with oil as above.

[7] #After overnight incubation with shaking at 30 °C, the emulsions were broken and the beads were washed 3□ with PBS-Tween (0.1%) followed by once with PBS-Tween containing a 10-fold molar excess of free MS2 coat protein (20 mg) for 15 min at room temperature.

[7] The reactions were incubated for 30 cycles of 94 °C for 1 min, 45 °C for 1 min, and 72 °C for 1 min.

[7] The PCR product was digested with Hind III and Sal I and ligated into the pAP-III6 vector for phage display.

[7] Phage displaying the scFvs were prepared by growth of aliquots of bacteria from the error prone PCR library and superinfection with helper phage.

[7] Immunotubes (Nunc Maxisorp) were coated with MS2 coat protein (10 mg/ml) overnight at 4 °C.

[7] After rigorous washing with PBS containing 0.1% (v/v) Tween 20, bound phage were eluted by addition of 0.5 ml of trypsin (1 mg/ml; Sigma–Aldrich, St. Louis, MO) in PBS.

[7] Infection was carried out for 30 min. at 37 °C without shaking.

[7] The following day, colonies were scraped into 7 ml of 2□YT containing ampicillin. Glycerol was added to a final concentration of 15% (v/ v) and the suspension stored at □80 °C.

[7] The samples were held at 37 °C without shaking for 30 min to allow phage infection and then centrifuged at 3000□ g for 10 min.

[7] The mixture was vortexed at top speed for 20 s to generate an emulsion.

[7] Average droplet size was estimated to be 100 lm using a hemocytometer.

[7] The emulsions were incubated in a 30 °C shaker overnight for phage production and capture on the beads.

[7] After overnight incubation, the emulsions were broken, the top [aqueous] layer containing beads was removed to a new tube, and the beads were captured on a magnetic separator.

[7] Once the OD600 reached 0.4, 25 ll aliquots of KM13 helper phage, each containing approximately 1 □ 109 virus particles, were added to each well to initiate superinfection.

[7] After a further 1 h growth, the plates were centrifuged at 1800□ g for 10 min and the supernatant aspirated from each well.

[7] The scFvs were expressed from the AP-scFv vector in Mach1 cells (Invitrogen, Carlsbad, CA) in low phosphate media (per liter: 3.57 g ammonium sulfate, 0.71 g sodium citrate dehydrate, 1.07 g potassium chloride, 5.36 g yeast extract, 5.36 g Sigma Hy-Case SF casein hydrolysate, 7 ml 1 M MgSO4, 14 ml 1 M glucose, pH 7.3 adjusted with KOH) supplemented with ampicillin [100 lg/ ml] for 20 h at 30 °C. Baffled flasks at a maximum 20% of flask volume were used to ensure good aeration (necessary to achieve phosphate depletion). The cultures were centrifuged and the pellets frozen at □80 °C.

[7] Cell pellets were resuspended in BugBuster (EMD Chemicals, Gib- bstown, NJ) supplemented with 5 lg/ml Benzonase (EMD Chemicals, Gibbstown, NJ), 1 mM PMSF, and protease inhibitor cocktail.

[7] The resuspended pellets were incubated with gentle shaking at room temperature for 20 min and then sonicated 3□ for 30 s each (with a 1 min pause between each pulse).

[7] Lysates were clarified by centrifugation at 15,000 rpm in a Sorvall SS34 rotor for 30 min at 4 °C.

[7] The scFvs were purified from the cleared lysates on HisPurTM Cobalt resin (Thermo Scientific, Rockford, IL).

[7] After binding the scFv proteins, the columns were washed with 50 mM Tris buffer pH 7.4 containing 250 mM sodium chloride, 10 mM imidazole, and 1 mM PMSF. The scFvs were eluted with 25 mM Tris, pH 7.4 containing 250 mM so- dium chloride, 500 mM imidazole, and 1 mM PMSF.

[7] Eluted protein was collected and analyzed by SDS–PAGE. [we... using ..technique]

[7] The column was equilibrated with 25 mM sodium phosphate, pH 7.0.

[7] The protein was bound to the column and eluted with a gradient of 0 to 300 mM NaCl in 25 mM sodium phosphate, pH 7.0. HiPrep 26/10 Desalting columns (GE Healthcare, Piscataway, NJ) were used for buffer exchange to PBS.

[7] ELISA was conducted on control and heated scFvs to determine the amount of binding following heating.

[7] The scFvs at 1.0 mg/ml in PBS were exposed to 70 °C for increasing amounts of time up to 1 h and then allowed to re-equilibrate to 4 °C on ice.

[7] ELISA plates were coated with 2 lg/ml MS2 coat protein followed by incubation for 1 h with a dilution series of the scFv.

[7] Binding was detected using anti-FLAG-HRP [Abcam].

[7] To test the stability of MS2, MS2-coated beads [7 □ 108 beads/ml] were heated in PBS-Tween [0.1%] to 70 °C for 15 min and then allowed to re-equilibrate to 4 °C on ice.

[7] Binding was detected using anti-FLAG-HRP [Abcam].

[7] For kinetic analysis, MS2 coat protein was coupled to a GLC sensor chip in a ProteOn system (BioRad, Hercules, CA).

[8] To control flow rates of three phases we use syringe pumps (Harvard Apparatus) and the flow is observed using an inverted microscope equipped with a high speed camera (Phantom V9.0).

[9] Monodisperse W/O/W double emulsions were prepared in glass microcapillary devices, which have been described previously.

[9] The round capillaries, with inner and outer diameters of 0.58 mm and 1.0 mm, were purchased from World Precision Instruments, Inc. and tapered to desired diameters with a micropipet puller (P-97, Sutter Instrument, Inc.) and a microforge (Narishige International USA, Inc.).

[9] These values were controlled by the size of the capillaries used and the flow rates of the different phases.

[9] A typical set of flow rates for the outer, middle, and inner phase was 3500, 800, and 220 μL/hr, respectively, and the droplet generation frequency was about 500 Hz.

[9] The formation of lipid vesicles was monitored with optical microscopy for samples placed between a coverslip and a glass slide separated by a 0.5 mm thick silicone isolator (Invitrogen, Inc.) alone or by two silicon isolators separated by an anodized alumina membrane filter (Anodisc 25, 0.2 μm, Whatman plc.).

[10] The surface tension of the dodecane-water interface was measured using a pendant drop method6 where a drop of the denser fluid, water, is suspended from a capillary tube in the lighter fluid, dodecane, containing the surfactant molecules.

[10] The time evolution of the surface tension depends on the material transport of the surfactant from the bulk to the surface.

[10] For systems where these assumptions are valid, eq 1 can be fit to the measured time dependence of the surface tension to determine the diffusion coefficient of the surfactant.

[10] This method has been used for systems such as surfactants and polymers to determine the time evolution of the surface tension, the diffusion coefficient of the surfactant molecule, its area at equilibrium, its affinity for the interface, the interaction between molecules at the interface, and the time scale for the rearrangement of polymer brushes at the interface.

[10] In the experiments reported here, the time evolution of the surface tension was measured by rapidly forming a 10 μL pendant drop at the tip of a vertical stainless steel needle [0.8 mm inner diameter] in a 10 mL rectangular glass cuvette containing lipid- saturated dodecane.

[10] Both phase-contrast images and images formed with crossed polarizers were acquired to determine the nature of the spontaneously formed particles.

[10] Briefly, CARS is a third-order nonlinear optical process in which a pump excitation field at frequency ωp and a Stokes excitation field at frequency ωs are mixed in a sample to generate an anti- Stokes signal field at frequency ωas] 2ωp - ωs. CARS microscopy provides a vibrational contrast based on the enhancement of the signal when ωp - ωs is tuned to a Raman active molecular vibration.

[10] Because CARS is a multiphoton process, the signal is generated only at the center of the focus, which permits three- dimensional sectioning of a sample.

C. Facilities/equipment

[3] The reactions were performed with Vent DNA polymerase (New England Biolabs) and the cDNA for either FR-a or FR-b as template.

[4] Sequencing of clones and expression constructs was performed on an ABI 3100 automated genetic analyser (Amersham Biosciences, Chalfont St. Giles, UK), using BigDye terminator chemistry, within our DNA Analysis Facility.

[9] Bright-field, phase-contrast, and fluorescence images were obtained with 5×, 10×, 20×, and 40× objectives at room temperature using an inverted fluorescence microscope [Leica, DMIRB or DMIRBE] or an upright fluorescence microscope (Leica, DMRX) equipped with a high speed camera (Phantom V5, V7, or V9) or a digital camera (QImaging, QICAM 12-bit).

[9] The process of lipid vesicle formation from double emulsions and the resulting lipid vesicles were imaged with a digital camera.

[10] The shape evolution of the drop was monitored and imaged every 5 s with a CCD camera. The contour of the drop was determined and the surface tension was automatically calculated by the instrument software [IT Instru- ments; Lyon, France] using the standard pendant drop analysis method.6

[10] Bright field microscopy observations of the dodecane suspensions were carried out with an inverted microscope (Leica) a few hours after injection of the water drop into the dodecane solution

[10] The composition of the onionlike structures formed during the spontaneous emulsification was determined using CARS microscopy.

[10] In our experiment, CARS images of the onionlike features were acquired by raster scanning two near-infrared picosecond laser beams.

[10] To study the kinetics of the spontaneous emulsification and the size of the particles formed, a pendant drop was formed by the same method as described for the surface tension measurements but in a 3.5 mL cylindrical glass vial placed in a light scattering instrument, an ALV DLS/ SLS-5000 spectrometer/goniometer (ALV-Laser GmbH, Langen, Germany).

[10] Dynamic light scattering [DLS] was used to determine the size of the small emulsion droplets formed spontaneously in dodecane.

D. Data analysis

[7] Data was analyzed by on board software or exported to Scrubber-Pro for analysis. The standard method of analysis utilized a 1:1 Langmuir model.

[10] The distributions of lipid, oil [fully deuterated dodecane], and water in the onions were mapped by tuning ωp - ωs to the CH stretching vibration at 2845 cm-1, the CD stretching vibration at 2125 cm-1, and the OH stretching vibration at 3445 cm-1, respectively.

[10] Acumulants analysis28 was performed on the initial decay to obtain the average size. For data taken during the first hour, this analysis provides a reasonable estimate, but after 2 h, the distribution became very polydisperse and a CONTIN fit was used to determine the particle size distribution

D. Results

A. Rehash literature review

[7] We have previously described a novel approach that utilizes water-in-oil (W/O) emulsions for the identification and isolation of cells secreting phage particles that display desirable scFvs.

[8] Microfluidic techniques provide a convenient method to make monodisperse hydrogel particles; this is accomplished by employing water-in-oil emulsion drops as templates to make hydrogel microparticles.

B. Rehash purpose

[2] To test the hypothesis that FRa translocates to the nucleus, a time course (0 min, 15 min and 30 min) for FRa nuclear localization was performed in DAOY cells treated with FA.

[3] To identify the sites of N-linked glycosylation in hFR-a and hFR-b, the putative modification

sites (Figure 1) were disrupted both individually and in all of the possible combinations.

[6] For this purpose, it is advantageous to employ a microfluidic device which allows exposure of the droplets to UV right after their formation.

[7] In the present study, we explore the potential of this technology for directed evolution experiments, including selection for tighter binding as well as resistance to elevated temperatures (i.e., thermal stability).

[8] By contrast, our approach enables the production of hydrogel microparticles dispersed directly in water; if necessary, the bilayer membrane can be removed by simple washing with distilled water.

[8] Therefore, this approach is potentially useful for encapsulation of live cells in hydrogel microparticles while retaining high viability; such cell-laden hydrogel microparticles have potential value for immunoisolation, drug delivery, and tissue engineering.

[9] Monodisperse double emulsions are generated with a glass microcapillary microfluidic device that combines a coflow and a flow focusing geometry shown in Figure 1a.

C. Rehash materials and methods

[1] Because of the low numbers of available tumors with less common morphologies, we compared serous tumors to all other morphologies combined, a design with 85% power to detect a difference of 20% in the proportion of patients with FRα-positive tumors.

[2] The ratio of the average band intensities of the two immunoreactive bands of FRa (42 kd and 38 kd doublet) with the marker of individual subcellular fraction (FRa/ICAM-1, FRa/hsp90, FRa/vimentin, FRa/pRB, and FRa/H3 bands) were determined using densitometry (Fig. 1d).

[2] To determine whether FRa activates FGFR4, FGFR4 promoter-luciferase constructs P-535/199 from human FGFR4 promoter were transiently transfected into DAOY cells, treated or not treated with FA.

[2] To confirm FRa binding to cis-regulatory elements of Hes1 and Fgfr4 promoters in intact embryos, chromatin immunoprecipitation (ChIP) experiments were performed using the lower lumbar region of the neural tube from wild-type (WT) mouse embryos (E10.0, 30 somite stage), an area where both of these genes are expressed. FRa bound to cis-regulatory regions of Hes1 and Fgfr4 promoters in vivo (Fig. 3a).

[3] Before the effects of the mutations in FR-a and FR-b on their cell surface expression and function were tested, it was desirable to ensure that the mutations did not affect the eciency of mRNA translation.

[3] Therefore FR-a, FR-b and their aglycosylated forms were synthesized by a translation system *in* vitro, analysed on denaturing electrophoresis gels and detected by autoradiography (Figure 3).

[4] By BLAST and keyword searching of the chick EST database (http://www.chickest.udel.edu/), we identified two cDNAs which appeared to code for members of the SULT1B and SULT1C families.

[4] First, we completely sequenced these cDNAs and the sequence data are shown in Fig. 1.

[5] As mentioned in Materials and methods, the only difference between the recombinant and wild-type polypeptides consisted in the N-terminal first amino acid residue which, for technical reasons, was an alanine in the recombinant chain.

[5] Samples from each step were collected and analysed by SDS-PAGE (Fig. 3).

[7] To test the hypothesis that phage micro-emulsion technology could be used to discriminate phage displaying scFvs with different binding affinities, we produced phage displaying either of two scFvs that each recognize the recombinant coat protein of the RNA bacteriophage, MS2.

[7] After overnight incubation of the emulsions at 30 °C for phage production and capture on the beads, the emulsions were broken and the beads were washed.

[7] The sorted phage were propagated and analyzed by ELISA against MS2 coat protein or the control protein, neutravidin, both before and after heating at 70 °C for 30 min.

[8] The Microfluidic device is comprised of two tapered cylindrical capillaries inserted in a square capillary whose inner dimension is slightly larger than that of outer diameter of the cylindrical capillaries, as shown in Figure 1.

[8] One circular capillary is tapered to have smaller orifice of 73 ìm in inner diameter and is treated with n-octadecyltrimethoxyl silane to make the surface hydrophobic; this is used for injection of the innermost aqueous phase: the hydrophobic surface prevents wetting of the aqueous phase on the outer wall of the capillary. The other circular capillary is tapered to have a larger orifice of 142 ìm in inner diameter and is treated with 2-[methoxy[polyethyleneoxy]propyl] trimethoxyl silane to make the surface hydrophilic; this is used for collection of double-emulsion drops: the hydrophilic surface prevents wetting of the middle oil phase on the inner wall of the capillary.

[8] As an innermost phase, we use 10 wt% or 15 wt% aqueous solution of poly[ethylene glycol]diacrylate [PEGDA, Mw 4000 g/mol] as an innermost phase of double-emulsion drops; we employ 10 wt% solution to study equilibrium states of polymersomes with hydrogel core, while 15 wt% solution to study dynamic behavior of swelling of the poly- mersomes or release of encapsulants.

[8] We inject the continuous phase, an aqueous solution of PVA, through the interstices of the collection and square capillaries at 3500 ìL/h as a counter flow to the innermost and middle phases.

[9] The inner phase [green arrow] is an aqueous solution of model encapsulant, while the outer

phase [blue arrow] is an aqueous solution of poly[vinyl alcohol] [PVA] and glycerol.

[9] The overall size and the thickness of the shell of the double emulsions can be adjusted by tuning the flow rates of each fluid phase and the diameters of each capillary in the device.

[10] The time evolution of the surface tension *γ[t]* of a dodecane-water interface during adsorption of POPS, a nonionic phospholipid, was measured for three different lipid concentrations at 25 °C.

[10] Using a micropipet, we injected a water droplet into a microscope chamber filled with dodecane containing POPC at a concentration of 0.25 mg/mL and imaged the contour of the droplet by optical microscopy.

[10] We investigated the chemical composition for POPC and POPS onions prepared at 27 °C and an initial lipid concentration of 0.25 mg/mL.

D. Results

- exhibit

[1] The primary tumors analyzed in this sample set are representative of the typical clinical presentation of epithelial ovarian cancer in the distribution of the stage at diagnosis, grade, and patient age (Tables 1 and 2); more patients with non-serous tumors were included than would be expected in the population.

[1] Serous tumors were more likely to be FRα-positive than all other morphologies combined (81.7% versus 59.8% positive, respectively, p b 0.001), with 66.7% of endometrioid (26 of 39) and 63.3% of clear cell (19 of 30) tumors and only two of nine mucinous tumors expressing FRα.

[1] FRα staining was evident in 109 of the 110 sections examined for these 18 patients.

[1] Specifically, of the three blocks available from her disease, there was strong staining in two sites, the ovary and the right paraaortic lymph node, but no staining in a biopsy of malignant material on the right pelvic peritoneum.

[2] The results of FRa immunoblots using mouse monoclonal antibody on nuclear extracts (Fig. 1a, b) showed that FRa translocates to the nucleus within 15 min of FA incubation.

[2] The results confirm that FRa binds Hes1 and FGFR4 promoters at AANTT or TTNAA and NTTTTN or NAAAAN sites.

[3] Wild-type hFR-a, containing three candidate N- glycosylation sites, and the mutant forms of the receptor, containing any one or two of these sites, showed multiple or diffuse bands, as expected, from microheterogeneity in N- glycosylation and the presence of incompletely modified intracellular protein in the cell lysates.

[3] Both the fully glycosylated FRs and the aglycosylated forms showed bands of similar intensities (Figure 3), indicating similar levels of translation for the two forms of the proteins.

[4] Phylogenetic analysis of this sequence (Fig. 2) indicated that the protein encoded by this cDNA belongs to the SULT1B subfamily, sharing around 60% amino acid sequence identity with mammalian SULT1B enzymes.

[4] Examination of the sequences showed they both contained the various SULT ‘‘signature’’ sequences, in particular PAPS binding elements.

[4] Pilot experiments with E. coli cell-free extracts showed that both chicken SULTs were able to sulfate the prototypical phenol sulfotransferase substrate 4-ni- trophenol (not shown).

[4] We demonstrated the presence of proteins in chicken liver, kidney, and brain cytosol fractions that cross-react with these antisera, and we are currently performing a detailed characterization of the SULT activities and isoforms present in these tissues.

[5] The structure of the plasmid used to transform P. pastoris cells and the deduced amino acid sequence from the cloned α' fragment subunit are shown in Fig. 1A and B, respectively. We show in figure 1...blablabla represent.

[5] Fig. 2 shows the electrophoretic analysis of the amplicons generated by PCR, to verify the integration of the construct into the plasmidial and yeast genomes.

[5] The size of the observed bands corresponded to that of the related genes in the respective samples, thus indicating that the insert is actually present in the transformed cells.

[5] The purification of tα' was achieved by a combination of precipitation steps and chromatographic approaches.

[5] Lanes 1 and 2 of Fig. 3 show the electrophoretic patterns of the yeast clones transformed with the empty and the integrated construct, respectively.

[5] As it is shown, a faint band at 26 kDa, indicating the expression of the recombinant polypeptide, was detected in the positive clone only.

[5] The effect of the purification steps on the homogeneity of the identified polypeptide is shown in Fig. 3.

[6] Working with a concentration above c* ensures that a space-filling polymer network can be formed inside each droplet, whereas keeping the concentration below ce* ensures that the viscosity of the solution is not too high.

[6] If the precursor chains have molecular weights of not more than about 500,000 g mol/1 (weight average), their microfluidic emulsification is highly controllable, whereas less control is achieved when chains with a higher molecular weight are emulsified.

[6] After forming pre-gel droplets, microgel particles are obtained by droplet gelation, achieved through photocrosslinking of the precursor polymers inside the drops (Fig. 1).

[7] Consequently, in each compartment, multiple copies of the recombinant phage are produced, some of which may bind to the target-coated bead.

[7] Kinetic analysis of AFX687 by SPR indicates that it has a binding constant (KD) of approximately 100 nM.

[7] The majority of the beads showed fluorescence above the background level, indicating that phage had bound, but the mean fluorescence intensity of the beads coated with AFX719-displaying phage was higher than the mean fluorescence intensity of the beads coated with AFX687-displaying phage.

[7] We show here that we can use phage micro-emulsion technology to distinguish two scFvs with a 300-fold difference in binding affinities (100 nM and 300 pM, respectively).

[7] In addition, we show that phage micro-emulsion technology can be used to select scFvs that are resistant to elevated temperatures.

[8] Therefore, resultant polymersomes can be collected at the bottom of the collection bath, and water flux through the bilayer membrane is eliminated.

[8] ... ; this expulsion is caused by an increase of the concentration of hexane in the oil layer; this is a poor solvent for PEG–b–PLA diblock-copolymer.

[8] Further reduction of the osmolarity to 0 mOsm/L induces significant increase of the diameter and, ultimately, rupture of all membranes as shown in Figures 3d,f.

[8] Once the membrane ruptures, sodium and chloride ions can freely diffuse into the hydrogel network.

[9] The uniformity in size and shape of the collected double emulsion drops, shown in Figure 1b, makes them ideal templates for the generation of uniform phospholipid vesicles.

[9] Phospholipid vesicles are obtained from the double emulsions by removing the solvent from the hydrophobic layer of W/O/W double emulsions (Scheme 1).

[10] Figure 1 shows the results plotted as a function of the square root of time, as suggested by eq 1.

[10] Phase contrast microscopy of the dodecane suspension collected after a few hours revealed the presence of two types of structures: submicron droplets, which were determined to be emulsion droplets, and larger droplets.

[10] Both of these structures were formed by spontaneous emulsification from the surface of the

water drop.

[10] These results are plotted in Figure 3A.

[10] In Figure 3B, the circles and triangles represent measurements performed for two distinct pendant drops under identical experimental conditions.

[10] Imaging with optical microscopy reveals the presence of spontaneously formed onionlike droplets.

[10] At even longer times, we observe a decrease of the scattering intensity and significant disparities between the mean droplet size measured for both samples.

[10] We found that this film is made of onionlike particles still tethered to the lyotropic film.

[10] We did not observe any shape fluctuations indicative of very low surface tension.

[10] Instead, we saw very distinct and well-defined droplets form on the oil side of the oil-water interface, as shown in Figure 4.

[10] We observed that the higher the concentration, the faster the growth of the droplets produced.

[10] In addition to vesicles, micron-sized oil droplets were also observed in the pendant water drop, as shown in Figure 7, for samples prepared between 27 and 30 °C only.

[10] This behavior was observed only for POPC.

[10] We have observed that for a given phospholipid, the initial lipid concentration and the ambient temperature set the morphology of the onions.

- discuss

[1] Therefore, this subset of patients with recurrent disease included both individuals with longer disease-free periods, good performance status, and localized disease and those with bowel obstructions or pain requiring palliative procedures.

[1] Interestingly, 2 patients who had tumors that were negative for FRα expression at diagnosis showed strong staining for FRα at recurrence, including one patient who had two subsequent debulking surgeries, both of which yielded tumors that were strongly positive.

[2] It is to be noted that a very faint band of immunoreactivity for FRa (38 kd band) was present in the nucleus even in the absence of FA.

[2] It is to be noted that all the membrane markers used here also showed strong immunoreactivity in the nuclear enriched fraction.

[2] In the absence of FA, FRa was predominantly present in the cytosolic fraction whereas in the presence of FA, the FRa (42 kDa band) appeared to translocate to the non-nuclear fraction (membrane, and cytoskeletal pellet fraction) and the 38 kDa band to the nucleus.

[2] When the data in Fig. 1e is presented as total non-nuclear fraction (membrane 1 cytosol 1 insoluble cytoskeletal pellet) and nuclear fraction (nuclear 1 chromatin bound) we observe that even in the absence of FA, FRa is present in the nucleus and in the presence of FA, there is a significant increase in the translocation of FRa to the nuclear fraction.

[2] This demonstrates that FRa activates FGFR4 promoter by binding to cis-regulatory elements.

[2] These data indicate that FRa transcriptional activation is not limited to FGFR4.

[3] A similar analysis of the wild-type and mutant constructs of hFR-b revealed multiple bands for wild-type hFR-b, the lowest of which corresponded to the aglycosylated form of the polypeptide (Figure 2C).

[3] Treatment of hFR-b with N-glycanase resulted primarily in a band corresponding to the aglycosylated form of hFR-b (Figure 2C).

[3] It is concluded that the two candidate sites of N-glycosylation in hFR-b are both modified *in* vivo.

[3] In general, the hFR-a constructs with three, two, one or no N- glycosylation sites showed progressively lower levels of [3H]folic acid binding at the cell surface (Table 2).

[4] It is interesting that both the chicken enzymes sulfate iodothyronines.

[4] This was somewhat surprising as the chicken enzymes both share approximately 60% amino acid sequence identity with their human counterparts.

[5] A relevant enrichment of the polypeptide was already achieved with the precipitation procedures, but further chromatographic steps removed the main contaminant proteins and allowed the recovery of the recombinant polypeptide in an almost homogenous form.

[6] The use of pre-polymerized precursors to form microgels provides a very useful benefit: since the particle gelation occurs independently of the polymer synthesis, microgels can be fabricated from highly pre-functionalized precursors.

[6] Apart from the production of isotropic, spherical microgels, micro!uidic emulsion templating can also serve to form particles with complex morphology.

[6] By this means, the use of macromolecular precursors allows not only microgels to be formed with precisely determined average degree of functionalization, but can also serve to control the spatial distribution of the functional sites across the microgel particles.

[6] By contrast, the pAAm core is always permeable and its degree of swelling remains

unaffected by temperature, thereby providing stability of shape.

[6] Thus, when the shells are swollen, the particles can be loaded with hydrophilic low molecular weight or mesoscopic additives.

[6] Upon increase of the temperature, the thermo-responsive shell collapses and encapsulates these actives in the pAAm core.

[6] Then, all surrounding feed material can be removed and the loaded particles can be stored at elevated temperatures.

[7] We believe that phage micro-emulsion technology provides numerous, significant advantages over current phage-display methodologies.

[7] For one, by keeping all of the phage copies in a single droplet, the signal of each clone is amplified.

[7] Second, the numbers of rounds of screening can be reduced to only one or two.

[7] Reducing the number of rounds of screening not only saves time, but it also eliminates the loss of diversity when one clone is amplified at the expense of others.

[7] In a typical phage display method, it is difficult to recover rare clones due to absorptive losses, and there is always clonal bias [and subsequent amplification] toward the strongest interactors.

[7] Because phage micro-emulsion technology amplifies the signals within a compartment, strong and weak interactors are no longer in competition for binding the target. For directed evolution of proteins, specifically in the affinity maturation of antibodies, reliable recovery of all improved rare mutants from a library is critical to rapid improvement.

[7] Another clear advantage of phage micro-emulsion technology is that the technique can permit discrimination between kinetic properties of binding a target in solution (i.e., on-rates, off-rates, dissociation constants, etc.).

[7] Generally, this is not the case in the simplest phage-display screening format, where the target is immobilized on the bottom of a microtiter plate well, and one isolates weak and strong binders together.

[7] Using micro-emulsion technology we can apply quantitative flow cytometric analysis to phage display, enabling the same kind of fine discrimination of kinetic parameters as has been achieved with yeast-display.

[8] Therefore, the middle oil phase dewets onto the surface of the innermost drop, forming a bilayer membrane as schematically illustrated in steps A–B of Figure 2a.

[8] Subsequently, the middle phase separates from the drop in about one minute, producing a

polymersome, a bilayer compartment of the innermost drop, as schematically illustrated in steps B–C of Figure 2a.

[8] After incubation of the polymersomes for 20 min at room temperature, we expose them to UV light with 2 W/cm2 in the collection bath for 30 s to fully polymerize the PEGDA prepolymers within the core; this leads the formation of hydrogel core in the interior of polymersomes, as schematically illustrated in steps C–D of Figure 2a.

[8] Although the osmolarity decreases as the PEGDA molecules are polymerized, the decrease is not significant because the number of molecular subunits, ethylene oxide, remains constant during polymerization;[7] thus, the osmolarity of the innermost phase, which originally contains 10 wt% of 4000 g/mol PEGDA, remains at approximately 100 mOsm/L.

??[8] Nevertheless, the dye molecules cannot penetrate through the bilayer wrapping the hydrogel core even though it is swollen, as shown in the confocal microscope images; all polymersomes do not allow penetration of the dye molecules in the aqueous solutions with 90 or 68 mOsm/L, while approximately 10% of the polymersomes permit penetration into their cores for the aqueous solution with 48 mOsm/L.

[8] In the aqueous solutions with 48 mOsm/L, polymersomes which exhibit increase of surface area less than critical value, approximately 40%, retain the dye molecules, while polymersomes which have large increase of surface area allow the diffusion of the dye through the resultant large gaps between amphiphiles.

[8] When they are transferred from 70 mM NaCl solution to distilled water, their diameter increases due to the inward flux of water through the bilayer membrane; nevertheless, there is no release of the encapsulants for a few minutes.

[8] However, the polymersomes ultimately swell enough that small gaps in the bilayer appear and release of the encapsulants begins.

[8] This occurs even before the hydrogel is fully swollen, as shown in series of confocal microscope images in **Figure 5a;** the first row [A1] shows release of FITC–dextran [Mw 20 000 g/mol] and the second row [A2] shows release of sulforhodamine B [Mw 558.7 g/mol].

[8] Although the bilayers maintain their integrity, intermolecular distance in the bilayers increases, enabling leakage of dye molecules through the bilayer; we estimate the critical value of increase of surface area for release of sulforhodamine B as approximately 40%.

[8] By plotting the time dependence of the diameter, we clearly observe the coincidence with the sudden release upon bursting of the membrane, as shown by the open cir- cles in Figure 5c.

[9] In the absence of phospholipids, the double emulsions are unstable, suggesting that phospholipids adsorb at the W/O and O/W interfaces and stabilize the structures.

[9] The reproducibility of the technique is further improved by carrying out the evaporation step

in highly concentrated glycerol solutions (typically above 80 wt %).

[9] When the double emulsion droplets wet the substrate, they can become pinned to it, and the inner drops can be released as vesicles into the continuous phase.

[9] Upon release of the inner drops, the middle organic solvent layer remains pinned to the substrate, as shown in the bottom panel of Figure 2.

[9]This process resembles a method where phospholipid-stabilized water droplets are formed in oil and subsequently transported through an oil/ waterinterfacethatiscoveredwithamonolayerofphospholipids, resulting in the generation of vesicles.

[9] In our case, the inner drops of the pinned double emulsion, stabilized by phospholipids, move across the interface between the oil and the continuous aqueous phase.

[9] Using the same approach, vesicles have been generated using a variety of phospholipids including both saturated (DPPC, DMPC, and DSPC) and unsaturated (DOPC and POPC) phosphocholines used alone or mixed with a phospho-L-serine (DPPS).

[10] However, at longer times the slopes of these curves change dramatically, indicating a slower decrease in surface tension with time, and an equilibrium value is not reached even after 6 h.

[10] This change in slope is reproducible, ruling out spurious effects such as those caused by dust in the sample.

[10] However, all of these values of the diffusion coefficient are about 2 orders of magnitude smaller than the value of D] 5 × 10-10 m2/s reported for the adsorption of lipid at air-water interfaces, suggesting that the evolution of the surface tension does not result from the simple diffusion of lipids to the interface.

[10] For these experiments, POPC was dispersed in dodecane at a concentration of 0.05 mg/mL, and the temperature was set to (30 (0.05) °C).

[10] Since these experiments were thermostated, convection currents were minimized and external perturbations of the pendant drop were eliminated.

[10] We measured the time evolution of the scattering intensity at 90° every 10 min for 6 h.

[10] In addition, we used DLS to measure the time evolution of the mean radius for these droplets; the results are plotted in Figure 3B.

[10] The kinetics of the spontaneous emulsification is controlled by the initial lipid concentration, which determines the number of lipid molecules reaching the interface per unit time to form the lyotropic phase, and hence the growth rate of the film; as the film gets thicker, the onion particles formed get larger.

- compare

[1] Similarly, when patients with fewer than 50% of malignant cells positive for FRα expression were grouped with the FRα-negative samples in order to assess the effect of the highest levels of FRα expression, no association could be found between FRα expression and time to recurrence as a univariate variable or after adjusting as above (data not shown).

[1] This is consistent with the effect of serous versus endometrioid tumor morphology on outcome in a study of stage III patients.

[3] Similarly, disruption of one of the two N- glycosylation sites in hFR-b resulted in cell surface FR that was 60–66% of the level of the wild-type protein (Table 3).

[3] In the absence of both the glycosylation sites in hFR-b, approx. 8 % of FR was expressed on the cell surface compared with wild-type FR-b.

[4] This is different from the human enzymes, since dopamine is not a substrate for either [HUMAN]SULT1B1 or [HUMAN]SULT1C2.

[4] Similarly, sulfation of iodothyronines by 1B1 (rat and human) and 1C1 (rat) and 1C2 (human) was very low, as it was with chicken SULTs 1B1 and 1C1.

[4] Therefore, the substrate specificity profiles for the chicken SULTs towards iodothyronines are broadly similar to the corresponding mammalian enzymes.

[5] The addition of tα' and the purified α' soybean subunit, as a positive control, to HepG2 cells produced a significant rise in LDL-R- mediated uptake and degradation compared to the untreated cells [Fig. 4].

[5] The result obtained with tα' at the highest concentration (8 μM) was similar to that of the second positive control, 1 μM simvastatin, i.e., +192% vs. +172% uptake and +143% vs. +150%, respectively.

[5] At no tα' concentration was there any evidence of cellular toxicity, as determined by the MTT and LDH assays (not shown).

[6] One of them uses microfluidic devices assembled from glass microcapillaries, and the other one uses devices stamped into silicone elastomers through the use of soft lithography.

[8] ....slightly higher concentration of ions in the continuous phase compared to that in the hydrogel network, the osmotic pressure difference decreases, thereby reducing the degree of swelling.

[8] By contrast, under the same conditions, normal polymersomes, containing unpolymerized 15 wt% PEGDA, exhibit rapid shrinkage with rupture of their membranes in just a few minutes, thereby releasing the encapsulants, as shown in Figure 4d.

[8] By contrast, the polymersomes without a hydrogel core immediately release their encapsulants as soon as they are dispersed into dis- tilled water, as shown in the bottom row [C] of Figure 5a.

[8] For comparison, we determine the time dependence of the diameter of the polymersome using [4A/π]1/2, where A is the area of the hydrogel core in the images in the third row [B] of Figure 5a.

[8] Inhibition of diffusion through the hydrogel is more effective when the size of encapsulants is closer to size of the hydrogel mesh.

[9] Such a dewetting phenomenon has also been observed when amphiphilic diblock copolymers are used for the generation of polymersomes from double emulsions.

[10] For times shorter than 30 min, the three curves decrease almost linearly with the square root of time; this time dependence is consistent with a diffusion-controlled adsorption mechanism.

[10] When imaged between crossed polarizers, as shown in Figure 2B, these droplets are clearly birefringent and have defects characteristic of molecules ordered normal to the interface, consistent with previous observations of multilamellar onionlike structures.

[10] Though the turbidity was more apparent at 20 °C, closer inspection reveals that over time the scattering intensity increases for all samples.

[10] In general, onions formed with POPS are smaller than 2 μm in diameter with a very small isotropic core that does not change significantly as we change the temperature.

[10] By contrast, onion structures observed for POPC are highly dependent on temperature and concentration.

- speculate

[2] These results suggest the following: (i) In the absence of FA, there is a more FRa in the cytosolic fraction; (ii) Upon FA treatment, FRa is distributed significantly to the non-nuclear membrane fraction as well to the nuclear enriched and chromatin bound fractions; (iii) Of the two immunostained FRa- 42 kDa and 38 kDa bands, the 42 kDa band seems to translocate to the membrane enriched fraction in the presence of FA.

[2] The above studies suggested that FRa translocates to the nucleus and in the presence of FA, it is enriched in the chromatin bound fraction.

[3] This result suggests that the transport of folate by FR is not dependent on the presence of N-glycosylation but is instead dependent on the expression level of [3H]folic acid-binding protein on the cell surface.

[3] From Tables 2 and 3 it is clear that the single conserved glycosylation site in hFR-a and hFR-b (Figure 1) is not the only site or even the most critical site at which N-glycosylation is required for the expression of functional FR.

[3] Although it seems that in the absence of glycosylation at position 161 in hFR-a, there is a greater decrease in the expression of FR, it might be concluded that, in general, every glycosylation site in both hFR- a and hFR-b contributes to the optimal expression of functional FR.

[4] this sequence contains the serine residue [Ser139 in SULT1B1 and Ser150 in SULT1C1] that is believed to play a critical role in PAPS hydrolysis.

[7] This data suggests that phage micro-emulsion technology is sensitive enough to discriminate high affinity binders from weaker binders.

[7] By varying the stringency of the wash conditions and using a less sensitive detection antibody, such as anti-FLAG, it may be possible to expand the dynamic range to discriminate antibodies with even smaller differences in affinity.

[7] By varying the stringency of the wash conditions and using a less sensitive detection antibody, such as anti-FLAG, it may be possible to expand the dynamic range to discriminate antibodies with even smaller differences in affinity.

[7] We can also incorporate a droplet generation device to control the uniformity of the droplets and the corresponding phage titers, since differences in phage expression could influence the signal on beads.

? spec or unsure/indeterminate ? [8] We attribute this very small decrease to the reduction of the osmolarity contrast between the interior and the exterior of the hydrogel by comparison with that of hydrogel in distilled water; because of the slightly higher

[8] We attribute this increased stability of the polymersomes with a hydrogel core to the formation of a hydrogel scaffold which supports the bilayer membrane; this prevents sharp undulation of the bilayer membrane during outward water flux, ensuring its integrity is maintained, as shown in Figures 4a–c.

[9] We believe glycerol plays an important role in reducing the line tension incurred in the solvent removal step;however, the exact stabilization mechanism is yet to be established.

[10] This suggests that large droplets [>2 *μm]* are sedimenting to the bottom of the vial thereby decreasing the intensity.

[10] These results provide strong evidence that the diffusion of water does not affect the spontaneous emulsification of water in dodecane.

[10] This strongly suggests that spontaneous emulsification results from the presence of lyotropic liquid- crystalline phases at the dodecane-water interface.

- explain

[1] Kaplan–Meier plots showed no statistically significant differences based on FRα status, although there was a modest trend towards earlier recurrence with FRα-positive tumors that was not reflected in the survival data (Fig. 3).

[4] We therefore propose that this sulfotransferase be named [CHICK]SULT1B1 under the proposed sulfotransferase nomenclature system.

[4] We propose that this sulfotransferase be named [CHICK]- SULT1C1.

[4] To study the properties of these chicken SULTs, we expressed the cDNAs in E. coli as previously described for various human SULTs.

[4] The data presented here suggest the chicken SULT1C1 is enzymatically more similar to rat SULT1C1 than to the human SULT1C2 isoform, a conclusion also supported by the phylogenetic analysis (Fig. 2).

[6] Both techniques offer versatile means to fabricate sophisticated channel geometries, and this operational feature opens a route to the formation of complex emulsion structures such as nonspherical droplets, anisotropic droplets, or multiple-emulsion "droplets-in-droplets".

[6] We use soft lithography to fabricate microfluidic devices from poly(dimethylsiloxane) (PDMS) which consist of a cross-junction channel to form drops and a basin channel to cure these drops.

[6] In addition, this strategy can be extended to the incorporation of many different functional sites by the use of several different precursor polymers, each imparting its own functionality.

[6] To demonstrate this concept, we synthesize pNIPAAm particles which contain defined amounts of two different fluorescent dyes, representing two different types of arbitrary functionalities.

[6] These "drops-in-drops" are useful to template coreeshell microparticles; in a typical experiment, we use them to form hollow gel shells.

[6] For this purpose, we use a glass microcapillary device to form monodisperse drops of a semidilute pNIPAAm precursor solution in a continuous phase of paraffin oil.

[6] To circumvent this limitation, we employ a step-by-step approach: first, we create monodisperse, micrometer-sized hydrogel particles which serve as the core material.

[6] In the first junction we add a semidilute, aqueous solution of crosslinkable pNIPAAm chains as the shell phase.

[6] In the second junction we add oil to form bi-layered pre-microgel drops.

[6] Due to this selective sensitivity, these particles are applicable for encapsulation and controlled

release purposes: when the pNIPAAm shell is swollen it is permeable, whereas it becomes impermeable when it collapses.

[7] To determine whether phage micro-emulsion technology could also be useful for selecting phage displaying scFvs with higher thermal stability, we first investigated the resistance of the M13 bacteriophage to elevated temperatures.

[7] After heating, the phage were tested for transducing ability by selection of infected cells on ampicillin-containing plates.

discuss[7] In fact, our data indicates that the phage remains infective after 10 h at 70 °C, and after several hours post incubation at 80 °C.

??[7] Because phage micro-emulsion technology amplifies the signals within a compartment, strong and weak interactors are no longer in competition for binding the target. For directed evolution of proteins, specifically in the affinity maturation of antibodies, reliable recovery of all improved rare mutants from a library is critical to rapid improvement.

[7] This application relies on the thermal resistance of the target protein or peptide.

[8] To explore the enhanced stability of the polymersomes which have the hydrogel core, we investigate their behavior when exposed to negative or positive osmotic pressures.

[8] As the osmolarity is lowered from 90 mOsm/L to 48 mOsm/L, the diameter of the polymersomes increases owing to the inward flux of water through the bilayer membrane caused by the higher osmolarity in the core, as shown in Figures 3a–c,f.

[8] Due to the inward water flux, the polymersomes are inflated and, finally, the molecular bilayer cannot cover the large surface area of the swollen hydrogel core; this results in rupture of the membrane, as shown in Figure S1 of the Supporting Information.

[8] The stability of polymersomes against such a positive osmotic pressure can also be enhanced by a hydrogel core.

[8] We summarize these three different release behaviors schematically in Figure 5b and in Movie S2 of the Supporting Information.

[8] We illustrate this by plotting time-dependence of the normalized fluorescence intensities of FITC–dextran or sulforhodamine B in the core of the polymersomes during the course of release in Figure 5c.

[8] This extended release of the dye molecules is a result of the hydrogel network, which limits the diffusion rates of dye molecules.

[8] Therefore, the retardation of the release is greater for FITC–dextran [Mw 20 000 g/mol] than for sulforhodamine B [Mw 558.7 g/mol]; it takes about 3 times as long, 440 s, to release of half of

their original FITC– dextran.

[8] Dumbbell-shaped polymersomes can encapsulate two different materials by enclosing each material in its own internal shell, thereby preventing mixing.

[8] ...; we attribute this to larger interaction parameters between these solvents and PEG than that between water and PEG.

[9] We use a mixture of volatile organic solvents, toluene and chloroform, to facilitate phospholipid dissolution and subsequent solvent evaporation.

[9] As the solvent layer becomes thinner during evaporation, the phospholipids are concentrated and then forced to arrange on the double emulsion templates, thereby forming vesicles.

[9] At the later stage of evaporation, the remaining solvent containing the excess phospholipids accumulates on one side of the vesicle, as shown in the top panel of Figure 2.

[9] Due to the fragile nature of the phospholipid bilayers, the vesicles often destabilize and rupture during the evaporation process.

[9] To avoid this, we find that slow evaporation of the organic solvent is critical; thus, we use a loosely sealed container to slow the evaporation.

?[9] Phospholipid vesicles can also be formed through another mechanism.

[9] This second route to phospholipid vesicle generation offers a simple and effective way of obtaining homogeneous vesicles if the double emulsions can be controllably pinned on a substrate.

[9] To demonstrate the high encapsulation efficiency of our approach, we encapsulate 1 μm yellow-green fluorescent latex microspheres inside phospholipid membranes which are labeled with a small amount (0.02 mol %) of Texas Red-labeled 1,2- dihexanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE).

[10] To examine whether the diffusion of water contributes to spontaneous emulsification, we prepared two samples: one with dodecane saturated with water and the other with dodecane containing 1% silicone oil, which prevents water from diffusing into the anhydrous dodecane.

[10] To monitor the appearance of the spontaneously formed submicron particles, we repeated the pendant drop experiment in a goniometer and used dynamic light scattering to measure their size.

[10] To investigate the impact of fluctuations, we imaged the surface directly with a microscope.

[10] To investigate the lyotropic semicrystalline films involved in the spontaneous emulsification process, we studied the particles produced at 35 °C for different lipid concentrations and compositions.

[10] To elucidate the influence of lipid packing properties on the spontaneous emulsification process, we measured the time evolution of the droplet size for two lipids that have the same acyl chains but different polar headgroups.

[10] More information about the microscopic composition of the interfacial lyotropic film can be obtained by examining the multilamellar onions that are produced from this film.

[10] To investigate the differences in microscopic composition, we used CARS microscopy

- exemplificate

[4] For example, the PSB loop, which contains the binding site for the 50- phosphate of PAPS, or 50PSB, is located towards the N-terminus of SULT proteins, and has a consensus sequence of TYPKSGTxW.

[4] For example, in rats SULTs 1A1, 1E1, 2A1, 2A2, and 2A3 did not sulfate any iodothyronines, whereas the human SULTS 1A1, 1A3, 1B1, and 1E1 are all active against various iodo-thyronines.

[6] In an illustrative example, we form microgels which are anisotropic and exhibit two distinguishable sides [“Janus microgels”].

[8] These polymersomes can encapsulate any water soluble ingredient in their gel cores; for example, polymersomes containing fluorescein isothiocyanate [FITC]–dextran molecules [Mw 20 000 g/mol] are shown in the confocal microscope images in Figures 2d,e.

[8] In this case, the osmolarity of the continuous phase has very little influence on the size of hydrogel core; for example, hydrogel cores which are transferred from distilled water into an aqueous solution of NaCl with 90 mOsm/L exhibit only a very small decrease in size as shown in Figures 3e,f.

[8] For example, when polymersomes with a hydrogel core, made by polymerization of 15 wt% PEGDA, are dispersed in an aqueous solution of NaCl with 250 mOsm/L, they remain as spherical in shape for several minutes and then shrink slowly without rupture of the membrane, retaining the encapsulated FITC–dextran molecules inside them, as shown in **Figures 4a–c.**

E. Conclusion

A. *Context*

Literature review

[1] Other groups have reported using PCR or radioligand binding assays to analyze FRα expression in ovarian cancer cell lines or small numbers of ovarian tumor specimens.

[2] Previous work from our lab demonstrated that in the absence of functional Pax3, FA increased KDM6B, through up-regulation of KDM6B targeting micro-RNAs. This in turn altered H3K27 methylation marks on the promoters of Pax3 downstream targets, Hes1 and Neurog2, and affected gene transcription14.

[2] Cazzaniga and colleagues compared levels of serum folate and assessed differences in folate binding ability with primary fibroblast cultures, from Alzheimer's disease (AD) patients and age-matched healthy subject.

[2] Circulating folate was significantly lower in AD patients, whereas folate binding to fibroblasts was significantly higher, possibly due to enhanced expression of FRa in AD fibroblasts.

[2] Grapp and colleagues reported that whereas WT FRa was localized in the plasma membrane, in cerebral folate deficiency FRa mutants were mistargeted to intracellular compartments.

[5] The cholesterol- and triglyceride-lowering capacity of soybean proteins has been demonstrated clearly. Soybean protein oral administration is currently the most potent dietary tool for treating hypercholesterolemic patients, thus providing a unique opportunity for the management of adults and very young subjects. Moreover, it is clearly established that plasma cholesterol reduction is greater in patients having a high baseline degree of cholesterolemia.

[5] The hypothesis that proteins *per se* reduce blood cholesterol arose from experimental studies indicating that a shift from animal to plant proteins in the diet activates the LDL-R system in the liver of laboratory animals, as well as in circulating lymphomonocytes of hypercholesterolemic patients.

[5] To identify the soybean protein components responsible for the cholesterol-lowering effect, in vitro studies were carried out with a human hepatoma cell line that is highly sensitive to factors regulating LDL-R expression and cholesterol biosynthesis/breakdown.

[5] We concluded that the purified α' subunit from the 7S soybean globulin up-regulated LDL-Rs in Hep G2 cells and confirmed this finding in cholesterol-fed rats.

[5] Although these data support the hypothesis that the protein moiety is responsible for the observed biological effect, arguments may be raised in favor of α' chain in vivo metabolic fate, since peptides and amino acids are normally produced by the action of gastric and/or intestinal proteolytic enzymes.

[5] However, an increasing number of animal and plant [poly]peptides are being claimed to play relevant regulatory functions, often attributed to anti-oxidant, antiproliferative and anti-inflammatory effects.

[5] As far as soybean is concerned, experimental evidence clearly indicates the possibility that peptides and even small compact proteins, such as the Bowman– Birk inhibitor, may be adsorbed, thus eliciting a number of effects, including anticancer, anti-inflammatory, radioprotective ones.

[5] Other peptides have been shown to exert hypotensive effects.

[5] Recently, an LDL-R transcription stimulating peptide [FVVNATSN], deriving from the 7S globulin à chain, has been identified from a soybean hydrolysate prepared by a protease from Bacillus amyloliquefaciens and then by chemical synthesis.

[5] In this case, an increased LDL-R transcription [+148%] was detected in Hep G2 cells exposed to the peptide at a concentration of 100 μM. Other peptides arising from the 11 S globulin have been shown to exert similar but lower activity.

B. *Purpose*

[1]The strengths of our study include the use of a large cohort of 186 patients with newly diagnosed, previously untreated ovarian cancer.

[2] In this study we have elucidated a second mechanism for FA action, through activation of FRa and its' subsequent action as a transcription factor.

Purpose with Results

[4] Here, we report the first detailed enzymatic and immunochemical analysis of small molecule sulfotransferases from the chicken G. gallus.

[4] The data presented on these important members of the SULT1 family clearly show that the avian and mammalian sulfotransferases are closely related structurally and functionally, and we have produced a set of tools with which to investigate further their role in chicken physiology.

[5] Therefore, in the search for a small-sized polypeptide, putatively responsible for the observed biological activity, we described in the present work the cloning, yeast expression and purification of a recombinant polypeptide which contained the N-terminal extension region of the soybean α' subunit and proved to be even more effective on LDL uptake and degradation than the full-length α' chain.

[8] In this work, we report a method to produce monodisperse polymersomes with a hydrogel core; these provide enhanced stability and sustained release of encapsulated materials.

[10] We have shown that when a water drop is placed in contact with a dodecane solution containing POPC or POPS, emulsification occurs spontaneously at the interface, creating either a water-in-oil emulsion or an oil-in- water emulsion.

C. *Materials and Methods*

[1] Another strength is the extensive follow-up available and complete clinical annotation of these specimens due to the retrospective nature of the study.

[1] We utilized formalin-fixed, paraffin-embedded tissues archived through the Mayo Clinic Tissue Registry, which has systematically preserved surgical specimens since the early 1900s.

[8] Polymersomes containing hydrogel prepolymers are prepared from templates of W/O/W double-emulsion drops;

D. *Results and discussion*

[1] Nevertheless, studies assessing FRα expression by other methodologies such as direct protein measurement, including quantitative detection of shed FRα in serum, may have different results.

[2] This model does not take into account FRa recycling and it is still unclear exactly how FRa translocates into the nucleus.

[5] The present results suggest that the amino acid sequence capable of inducing the biological response lies in the N-terminal extension domain of α' chain.

[5] Moreover, we found that the truncated polypeptide exerts its effects at concentrations in the order of magnitude as those of simvastatin, a potent hypolipidemic drug.

[6] The combination of microfluidic droplet templating with subsequent polymer-analogous droplet gelation offers promising means to form functional microgel particles with independent control of their morphology and chemical composition.

[8] ...subsequent photo-polymerization of the prepolymers creates a hydrogel network in the interior of the polymersomes.

[8] Accordingly, polymersomes with a hydrogel core can encapsulate and deliver active materials in a more safe and precise fashion, preventing undesired rupture of the bilayer and providing controlled rate of release.

[8] In addtition, these polymersomes can serve as templates to produce hydrogel particles direcly in water, enabling safe embeding of cells or other water-soluble materials in hydrogel microparticles.

[10] The spontaneous emulsification of the interface explains the apparently slow dynamics of lipid adsorption at the oil/water interface. It also accounts for the difficulty experienced in controlling the size of inverted emulsions produced by shear or mixing.

[10] In addition to POPC and POPS, we have observed spontaneous emulsification with other phospholipids such as 1,2-dioleoyl-sn-glycero-3-phosphocholine [DOPC] and 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine [SOPC], and the phenomenon is certainly more general than these four lipids.

[10] We have identified the mechanism as being due to formation and swelling of the liquid-crystalline lipid structures.

[10] Lipids can self-assemble over several layers at an oil-water interface forming a thick film, which seems to be responsible for the formation of the multi- lamellar onions observed in the oil

phase.

E. *Conclusion*

- present research limitations

[1] A limitation to the study is that FRα expression occurs frequently, especially in the common high-grade, high-stage serous tumors that are most likely to recur.

[1] In addition, because the less common morphologies that ultimately showed less FRα expression were oversampled, this retrospective study cannot be used to establish the prevalence of FRα+ tumors in ovarian cancer patients; in other words, our observed proportion of FRα-positive tumors of 72% is likely an underestimate of true prevalence.

[5] How this effect could be achieved in vivo is difficult to establish as yet and this will require further investigations.

[5] However, in vitro interaction between the 7S soybean globulin and thioredoxin, a small multifunctional protein with a redox-active disulfide-dithiol in the conserved active site sequence Cys-Gly-Pro-Cys, has been shown.

[6]However, despite extensive use, these existing techniques of microfluidic particle formation have an intrinsic limitation: since the microfluidic templating and the subsequent polymerization are coupled within one single step, independent control of both the material properties and the morphology of the resultant microparticles is limited.

- implications of the research

[1] In summary, the common expression of FRα on primary and synchronous metastatic disease as well as on recurrent disease suggests that FRα-based therapeutic strategies may be helpful for most women with ovarian cancer, whether newly diagnosed with disseminated disease or experiencing disease recurrence.

[1] A number of strategies to take advantage of this observation are being pursued, with several folate-targeted drugs currently undergoing testing in clinical trials.

[2] This novel role of FRa as a transcription factor provides insight into developmental mechanisms associated with FA responsiveness.

[2] It also provides an exciting new avenue to explore for treatment of diseases associated with FA deficiency, FRa misregulation and cancers which express FRa as a biomarker.

[3] It should be noted that, for many proteins that in the absence of N-glycosylation are retained inside the cell, there are no practical means of monitoring folding in intracellular compartments.

[3] An obvious implication of this finding is that core glycosylation is not likely to be involved in

a specific step in the folding pathway of the polypeptide backbone of FR but, rather, glycosylation might facilitate the folding of FR in a general manner, either by a direct effect on the physical properties of the polypeptide or by indirect means.

[5] This finding might explain the longer lag phase of LDL oxidation induced by cupric oxide observed in rabbits fed cholesterol-rich diet containing soybean protein vs. that found in rabbits fed the same diet but containing casein as protein source.

[5] The data obtained in the present study are intriguing because they show for the first time that a truncated recombinant form of soybean 7S globulin α' chain is active in an in vitro model at concentrations [b10 μM] that are similar to those reported for simvastatin.

[5] Moreover, the use of a recombinant protein rules out any involvement of other protein and nonprotein soybean components, including isoflavones, for which clear benefits have not been claimed.

[6] This approach separates the particle formation from the synthesis of the polymer material and allows each to be controlled independently; it thus combines the control of microfluidic templating with the flexibility of preparative polymer chemistry.

[7] We have shown that the phage are completely resistant to heating at 50 °C for at least 16 h.

[8] Therefore, this novel approach to make a hydrogel network in polymersomes will provide new oppotunities for a wide range of encapsulation and delivery applications of active ingredients such as drugs, cosmetics, nutrients, and cells.

[9] In conclusion, we present a general method for fabricating monodisperse phospholipid vesicles using controlled double emulsions as templates.

[10] The study of these inverse onions with CARS microscopy shows that variations in composition are related to changes in hydration of the lipid bilayers due to the lipid polar head due to temperature changes.

- make recommendations/future research

[1] Prospective studies of FRα expression and its association with outcome would be required to confirm these results.

[2] However, up-regulation of Hes1 or Neurog2 in FA-rescued Sp2/2 embryos14 suggests that FRa may also have a role independent of Pax3. Future work shall test this hypothesis.

[2] Further work needs to be done to examine direct transcriptional activation of FRa responsive genes by FRa and its' role in these multifactorial diseases.

[3] Further, the effects of this...can be studied.

[3] These observations, together with the finding that the expression of hFR-b can be further enhanced by the introduction of an additional unnatural site of N- glycosylation, provide strong evidence that it is the total mass of N-glycosylation rather than any specific site of the modification that is critical for the proper folding of FR.

[5] Studies are in progress to identify the minimally active region[s], by means of further reducing the size of the truncated form, and to trace the metabolic pathway of the properly labeled polypeptide in target cells.

[5] In addition, in order to reveal the potential lipid-lowering properties of the recombinant polypeptide, experiments will be performed in rats fed a cholesterol- rich diet, an animal model of human hypercholesterolemia.

- present contributions / value of the research

[2] The observation that FRa acts like a transcription factor is relevant to our understanding of the mechanisms of FA action during development and has significant implications for disorders associated with FA deficiency and FRa misregulation and for management of human cancers which express FRa as a tumor antigen.

[2] The data presented in this paper provides relevant insight to these clinical situations. If FA interaction with FRa is misregulated, key transcriptional events may be affected.

[2] This in turn can lead to a series of developmental consequences or to adult onset disease associated with FA levels.

[2] In summary, our study shows that FRa is localized in the nucleus, where it binds to cis-regulatory elements (AANTT or TTNAA and NTTTTN or NAAAAN) on FA modulated genes and activates their transcription.

[4] The data presented on these important members of the SULT1 family clearly show that the avian and mammalian sulfotransferases are closely related structurally and functionally, and we have produced a set of tools with which to investigate further their role in chicken physiology.

[3] The results of this study have clearly demonstrated that that N- glycosylation at only the single conserved site in FR will not result in optimal expression of [3H]folic acid-binding protein.

[5] The results of these studies could lead to the development of functional foods with beneficial effects on various diseases, including hyperlipidemia and cardiovascular disease, to be used alone or in combination with drugs in lipid-lowering therapies.

[6] These materials are useful for various applications, such as the encapsulation and controlled release of actives.

[6] If the implementation of the techniques presented in this paper is achieved through the use of

microfluidic devices made by soft lithography, the microgel fabrication can be scaled up by device parallelization, offering the potential to produce larger quantities of sensitive microparticles.

[7] In addition, we show that phage micro-emulsion technology can be used to select scFvs that are resistant to elevated temperatures.

[8] Moreover, the hydrogel core can serve as a model cytoskeletal element when the polymersomes are employed as artificial system to study fundamental cell functions.

[9] Our simple and versatile technique offers a novel route to generate monodisperse phospholipid vesicles with high encapsulation efficiency for biomedical applications and for fundamental studies of biomembrane physics.

[10] These results confirm that the microscopic organization of the particles formed is dictated by the hydrophilic lyophilic balance of the lipid used at the experimental temperature and by the lipid initial concentration in dodecane.

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G. Caption

FIGURE

[1] Fig. 1. Weak, moderate, and strong FRα expression. Shown are representative cores of ovarian cancer tissues on TMAs that were scored with weak (A), moderate (B), and strong (C) FR! expression after immunohistochemistry using FBP343 antibody. Note that the tumor stroma is negative and malignant epithelial cells are largely positive.

[1] Fig. 2. FRα expression in primary and recurrent samples. The frequency of FRα expression in matched tumor samples from an initial diagnostic surgery and subsequent secondary debulking surgery for disease recurrence is shown. Examples of four patients representing each of the expression patterns are shown.

[1] Fig. 3. Kaplan–Meier analyses of recurrence-free and overall survival. Recurrence- free survival (A) and overall survival (B) in patients with FRα-positive (thick line) and FRα-negative (hatched line) ovarian tumors.

[5] Fig. 1. [A] Overview of the pPICZ!B-tα construct. Expression of tα' is driven by the alcohol oxidase [AOX] methanol-inducible promoter [5'AOX1]; the α-mating factor [α-MF] promotes secretion of the recombinant protein to the medium; AOX1 TT: AOX transcription termination region. The *Sh ble* gene confers resistance to zeocin; pUC Ori: origin of replication for the high-copy-number plasmid in E. coli. The other abbreviations refer to the cleavage positions of restriction enzymes; bp: base pair. [B] Deduced amino acid sequence of the recombinant polypeptide [tα'].

[5] Fig. 2. Agarose electrophoretic gel of transformed *P. pastoris* amplicons. Lane M: 1-kb DNA ladder marker; Lane 1: genomic DNA wild-type X33 strain; Lane 2: genomic DNA clone X33-pPICZαB-tα'; Lane 3: plasmidial DNA pPICZαB-tα'; Lane 4: plasmidial DNA pPICZαB [empty construct]. Arrow a: AOX1 gene; arrow b: amplicon including tα' recombinant gene; arrow c: amplicon including the empty construct.

[5] Fig. 3. SDS-PAGE under reducing conditions of P. pastoris culture media [A] and tα' purification steps [B]. [A] Lane 1: X33-pPICZαB [empty construct] induced with 1% of methanol; Lane 2: X33-pPICZ!B-tα' induced with 1% of methanol. [B] Lane 1: Acetone powder; Lane 2: DEAE-cellulose unbound fraction; Lane 3: DEAE-cellulose 150 mM NaCl eluted fraction; Lane 4: DEAE-cellulose 250 mM NaCl eluted fraction.

[5] Fig. 4. Effect of tα' and full-length α' soybean subunit on the LDL uptake and degradation by HepG2 cells. Confluent monolayers of HepG2 cells were preincubated at 37°C for 24 h in MEM with 5% LPDS, in the presence of purified α' subunit [α'], the recombinant polypeptide [tα'] or simvastatin [S] at the concentrations indicated in the figure. L: LPDS as the control. After the addition of ^{125}I-LDL, cells were incubated for an additional 5 h and then analyzed as described in Materials and methods. The data are means±S.D. of three independent experiments, each performed in quadruplicate. ∗Pb.05 vs. LPDS and ∗∗Pb.001 vs. LPDS.