Fusion-Inducing Liposomes for Efficient Intracellular Delivery: Continuum Models and Experiments

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Research and education activities (Year 1)

Students: 1 undergraduate student, 3 graduate students and 1 postdoctoral researcher have been involved in Year 1 of the grant.

- ◇ Undergraduate student:
  - Quang Hoang.
- ◇ Graduate Students:
  - Yerbol Palzhanov,
  - Qi Sun,
  - Alexander Zhiliakov.
- ◇ Postdoctoral Researcher:
  - Michele Girfoglio.

Zhiliakov participated in two conferences (SIAM TX-LA Section Annual meeting in 2020 and SIAM CSE 2021). Zhiliakov has been offered an Animation Technology Internship at Walt Disney Pictures for Summer 2021.

Research and education activities (Year 1)

- **Research Presentations:** Quaini presented the research related to this NSF proposal at 5 conferences and seminars: Invited talk at ICERM workshop on Algorithms for Dimension and Complexity Reduction (online, 03/23-27/20), Invited talk at AMS Fall Central Sectional Meeting 2020 (online, 09/12-13/20), Invited talk at World Congress on Computational Mechanics (online, 01/11-15/21), Seminar for the Department of Mathematics at Friedrich-Alexander-Universität Erlangen-Nürnberg (online, 02/01/2021), Invited talk at 2021 SIAM Conference on Computational Science and Engineering (online, 03/01-05/21). Olshanskii presented the research related to this NSF proposal at Comput. Math. and Applications Seminar of Math. Institute, Oxford (Oct. 2020; online); Invited talk at SIAM TX-LA meeting (Oct. 2020; online).

Research and education activities (Year 1)

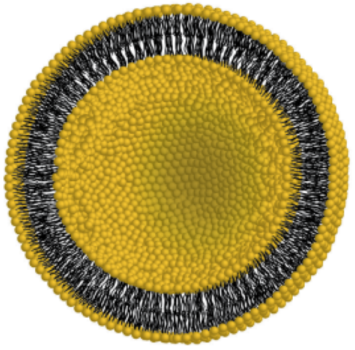
- **Courses for graduate and undergraduate students:** In the fall semesters 2020 and spring semester 2021 Quaini has taught Numerical Analysis for graduate students. In the fall semester 2020 Quaini has taught Linear Algebra with Matlab. Topics of this project were mentioned as examples in this course to motivate the students.
- **Organization of Conferences and Workshosp:** Quaini is co-chairing with Dr. Rozza (SISSA, Italy) the International Workshop on Reduced Order Methods, to be held at the National University of Singapore in 2023.

Research and education activities (Year 1)

- **Organization of Mini-Symposia:** Topics from this project have been or will be presented at the following Mini-Symposia organized by Quaini: "Recent Advances in Scientific Computing and Applications" at AMS Fall Central Sectional Meeting (online, 09/12-13/20), "Advances in Intrusive and Non-intrusive Techniques in Reduced Order Modelling for Flow Analysis, Control and Optimization" at ECCOMAS 2020 (online, 01/11-15/21), "Reduced Order Methods for Parametric CFD Problems" at SIAM CSE (online, 03/01-05/21), "Advances in Coupled Model Reduction in Heat Transfer, CFD and FSI" at COUPLED PROBLEMS 2021 (online, 06/13-16/21), "Model reduction and machine learning for fluids and fluid-structure interactions" at the Mechanistic Machine Learning and Digital Twins for Computational Science, Engineering and Technology (San Diego, 09/23-26/21).

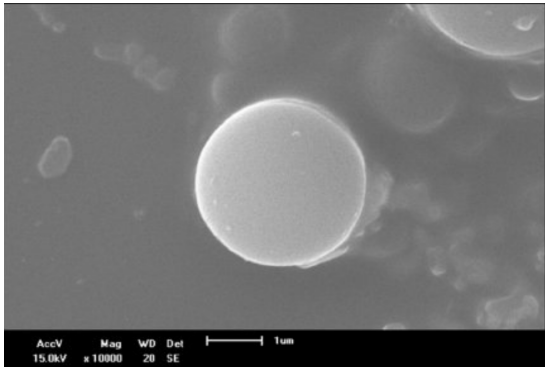
Motivation

Liposomes (typical diameter <100 nm) are lipid vesicles with a bilayered membrane structure.



Liposomes are considered to be the most successful drug carriers.

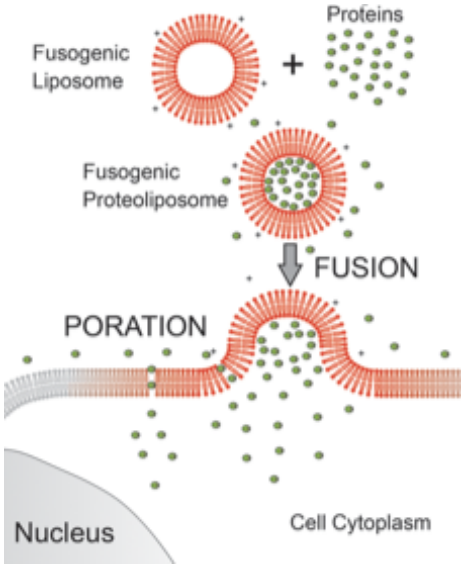
Despite extensive research, only a few liposomal drugs have been approved by the U.S. Food and Drug Administration.



Key liposomal characteristics are

- high target selectivity
- enhanced target cell uptake
- limited toxicity

A promising class of liposomes



Fusogenic liposomes are liposomes formulated to facilitate fusion.

Membrane fusion provides a neat strategy for delivery of therapeutic molecules across the cellular plasma membrane into the cell interior.

Fusogenic liposomes successfully deliver biomolecules into cells. However, the fusion-inducing components needed for efficient delivery make these liposomes toxic in vivo.

One needs to find a balance between fusogenicity and toxicity.

Possible solution: low concentrations of fusogenic lipids that can be presented in dense patches through phase separation.

Our goal: to apply complementary mathematical, computational, and experimental tools to design and develop a new class of liposomal carriers, called patchy fusogenic liposomes.

Lateral phase separation with conservation

Conservation law for representative concentration c on stationary $\Gamma \subset \mathbb{R}^3$:

$$\rho \frac{\partial c}{\partial t} + \operatorname{div}_\Gamma \mathbf{j} = 0$$

ρ: total density of the system

$\mathbf{j} = -M \nabla_\Gamma \mu$: diffusion flux (Fick's law, empirical)

$M = M(c)$: mobility coefficient

$\mu = \frac{\delta f}{\delta c}$: chemical potential

$f(c) = f_0(c) + \frac{1}{2}\epsilon^2 |\nabla_\Gamma c|^2$: total specific free energy (Ginzburg-Landau potential)

In order to have phase separation, f_0 must be a non-convex function of c.

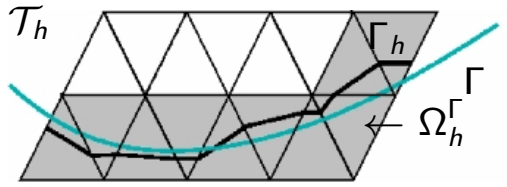
Surface Cahn–Hilliard equation

$$\rho \frac{\partial c}{\partial t} - \operatorname{div}_\Gamma \left(M \nabla_\Gamma \left(f_0' - \epsilon^2 \Delta_\Gamma c \right) \right) = 0 \quad \text{on } \Gamma$$

TraceFEM: basic principles

We study for the first time a geometrically unfitted finite element method for the Cahn–Hilliard equation

2D illustration



Idea

Use a trace space induced by FE functions for the bulk triangulation $\mathcal{T}_h$.

Define

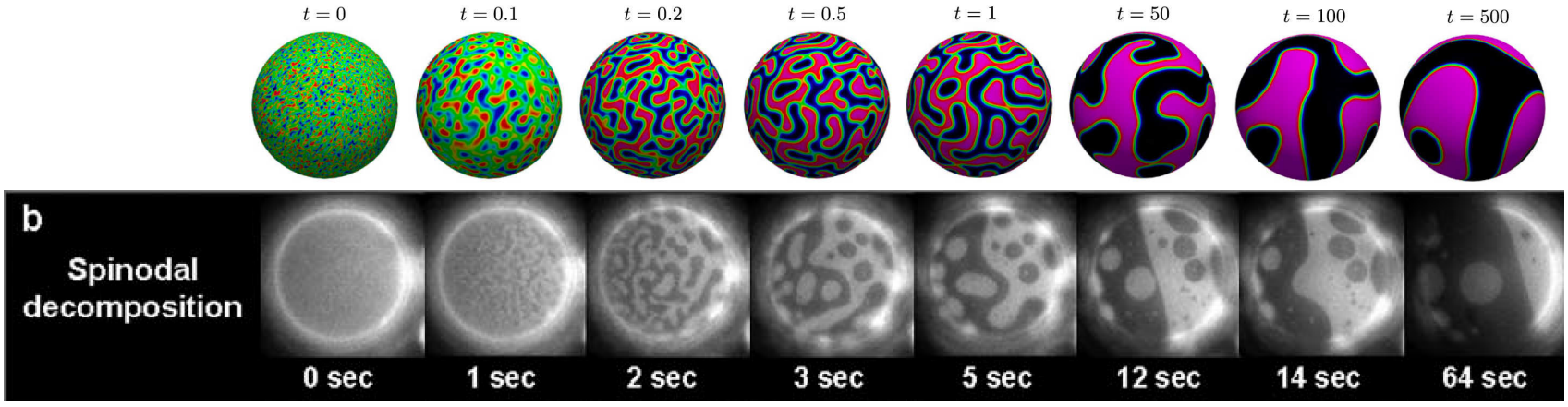
Define the outer space: $V_h = \{v \in C(\Omega_h^\Gamma) : v \in \mathbb{P}_1(T) \text{ for any } T \in \mathcal{T}_h^\Gamma\}$

And then define the trace space for V_h:

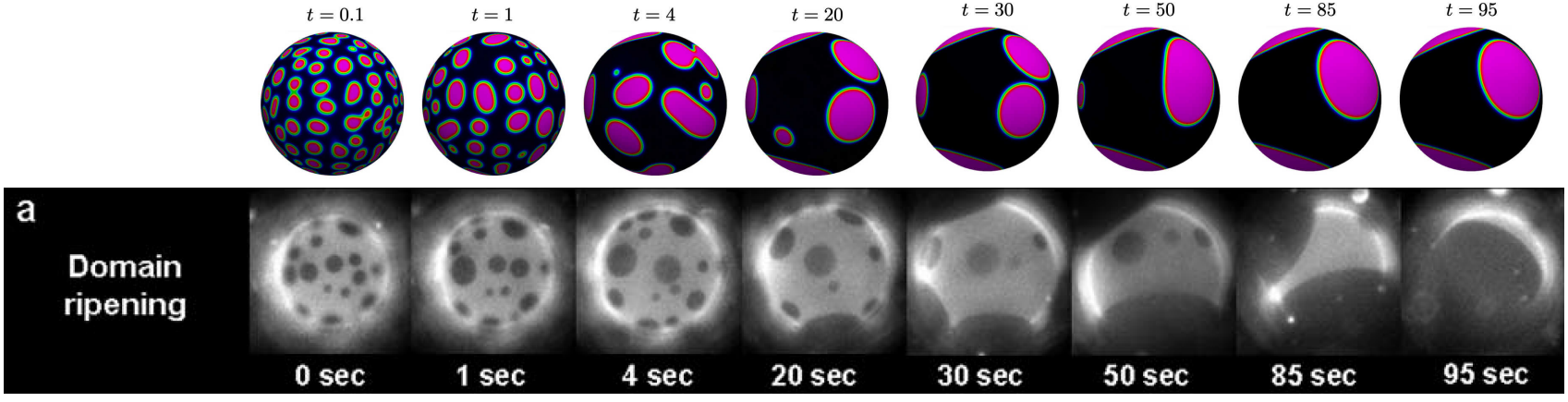
$$\text{interface FE space } V_h^\Gamma := \{\psi_h \in C(\Gamma_h) : \exists\, v_h \in V_h : \psi_h = v_h|_{\Gamma_h}\}.$$

Qualitative comparison with experiments

Two-component mixture with random initial condition (1:1), $\epsilon = 0.01$ (realistic value)



Two-component mixture with random initial condition (2:1), $\epsilon = 0.01$



Advantages of TraceFEM

- Surface Γ is not meshed directly.
- Number of active degrees of freedom is optimal, it is comparable to methods in which Γ is meshed directly.
- Optimal order of convergence in L^2 norms.
- Amenable to both space and time adaptivity.
- Effective condition numbers of matrices are comparable to common FEMs.
- If Γ evolves, Γ is not tracked by a mesh (Eulerian method).
- If Γ evolves, one recomputes matrices using the same data structures.

Lateral phase separation on an evolving surface

Using elementary tangential calculus, we derive a Cahn–Hilliard problem posed on an evolving material surface:

$$\dot{\rho} + \rho \mathrm{div}_\Gamma \mathbf{u} = 0 \quad \text{on } \Gamma(t)$$

$$\dot{c} - \rho^{-1} \mathrm{div}_\Gamma \left(M \nabla_\Gamma \left(\frac{1}{\epsilon} f_0' - \epsilon \Delta_\Gamma c \right) \right) = 0 \quad \text{on } \Gamma(t)$$

where $\dot{f}$ is the material derivative of f.

- The system is one-way coupled
- We are not aware of a minimization property for the Cahn–Hilliard problem in time-dependent domains $\rightarrow$ the system is no longer dissipative

Evolving surface: weak formulation

To be able to use an Eulerian approach, we introduce an arbitrary smooth extension of c and μ to a neighborhood

$$\mathcal{O}_\delta(\Gamma(t)) = \{\mathbf{x} \in \mathbb{R}^3 \,:\, \mathrm{dist}(\mathbf{x}, \Gamma(t)) < \delta\}.$$

and reformulate the problem in terms of the extended variables.

The weak formulation becomes:

$$\int_{\Gamma(t)} \rho \left(\frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c \right) v \, ds + \int_{\Gamma(t)} M \nabla_\Gamma \mu \, \nabla_\Gamma v \, ds = 0,$$

$$\int_{\Gamma(t)} \mu \, q \, ds - \frac{1}{\epsilon} \int_{\Gamma(t)} f_0'(c) \, q \, ds - \epsilon \int_{\Gamma(t)} \nabla_\Gamma c \, \nabla_\Gamma q \, ds = 0,$$

$$\frac{\partial \mu}{\partial \mathbf{n}} = \frac{\partial c}{\partial \mathbf{n}} = 0 \quad \text{in } \mathcal{O}_\delta(\Gamma(t)),$$

for all smooth v and q defined in $\Gamma(t)$.

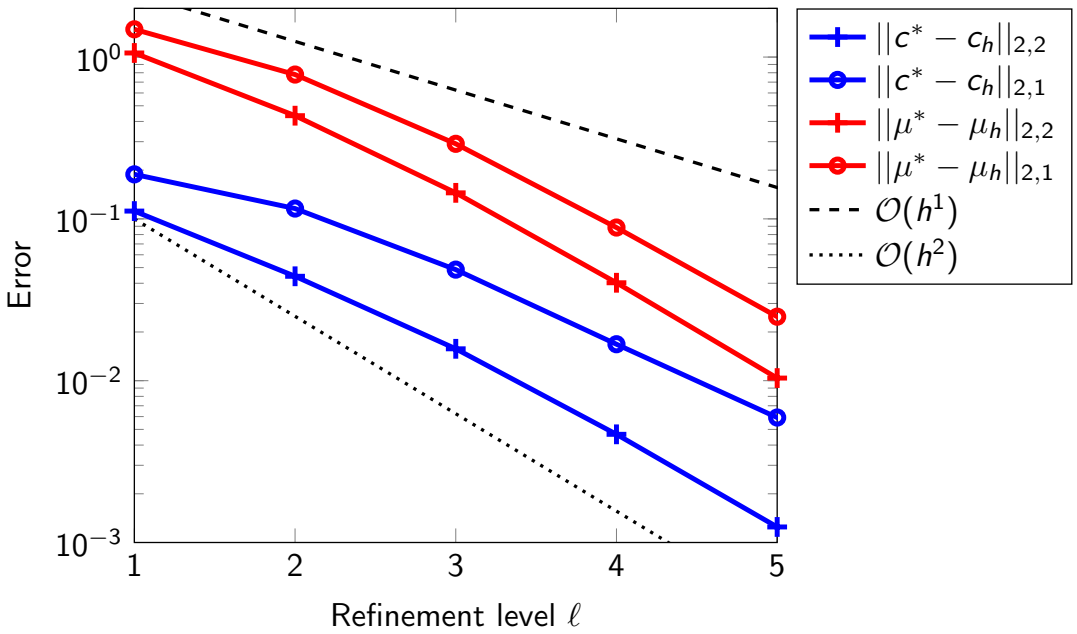
We discretize first in time (semi-implicit Euler method) and then apply TraceFEM for space discretization. Everything else is like the stationary surface case presented in [Yushutin-Q.-Majd-Olshanskii, *IJNMBE* 2019]

Simple motion

We consider the following synthetic solutions

$$c^* = \frac{1}{2}\left(x_1 x_2 + 1\right), \quad t \in [0, 0.1]$$

on a unit sphere that is translated and rotated, with $\epsilon = 0.1$, $h_\ell = \frac{10/3}{2^{\ell+2}}$, $\Delta t = 4^{1-\ell}/10$.



Oscillating ellipsoid

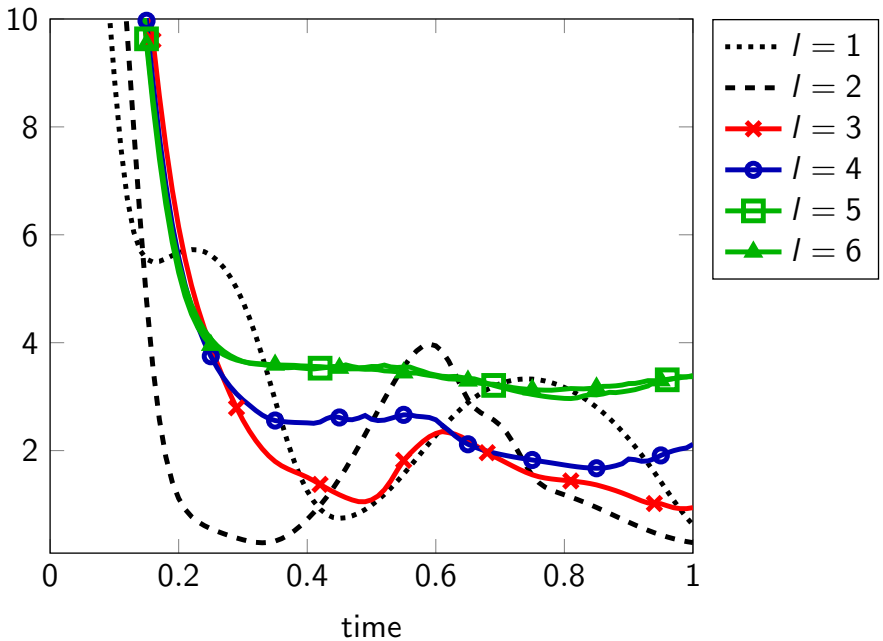
We consider time-dependent surface $\Gamma(t)$ to be an oscillating ellipsoid. As initial solution, we take:

$$c_0 = 0.5 + 0.05\cos(2\pi x_1)\cos(2\pi x_2)\cos(2\pi x_3).$$

We set $\epsilon = 0.01$, $h_\ell = \frac{10/3}{2^{\ell+2}}$, $\Delta t = 0.01$.

Discrete Lyapunov energy:

$$E_h^L(c_h) = \int_{\Gamma_h} f(c_h) ds$$



Colliding spheres with pre-separated phases

The evolving surface is the zero level set of level set function

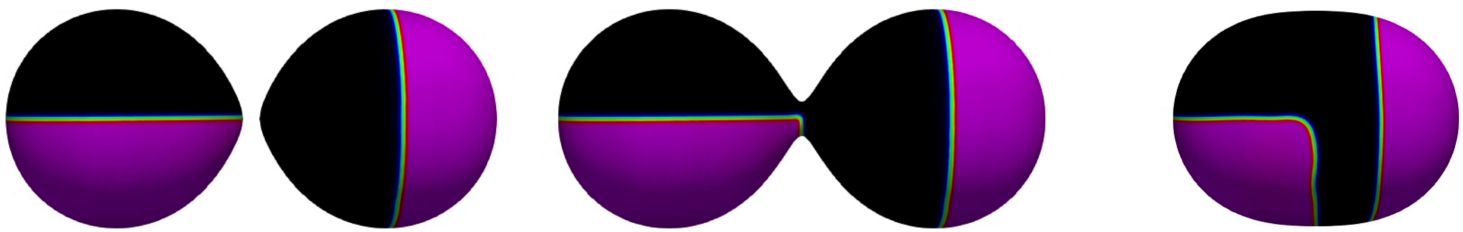
$$\phi(\mathbf{x}, t) = 1 - \frac{1}{||\mathbf{x} - \mathbf{x}_c^+(t)||^3} - \frac{1}{||\mathbf{x} - \mathbf{x}_c^-(t)||^3},$$

with

$$\mathbf{x}_c^{\pm}(t) = \pm \left(\frac{3}{2} - t, 0, 0 \right), \quad t \in [0, 1.5].$$

We take an initial solution with pre-separated phases on each ball.

$t = 0.23$ $t = 0.25$ $t = 1$



Pattern formation on colliding spheres

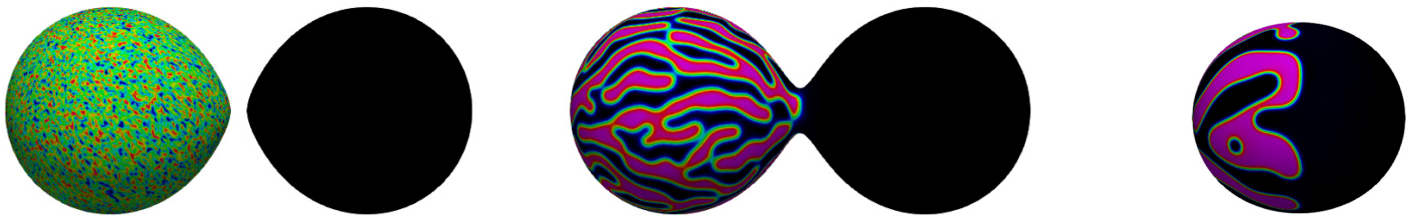
Same surface dynamics as before, but different initial condition:

- two-component mixture with random initial condition (1:1) for the ball initially centered at $\mathbf{x}_c^-(0)$
- homogeneous phase for the ball initially centered at $\mathbf{x}_c^+(0)$

We set $\epsilon = 0.01$.

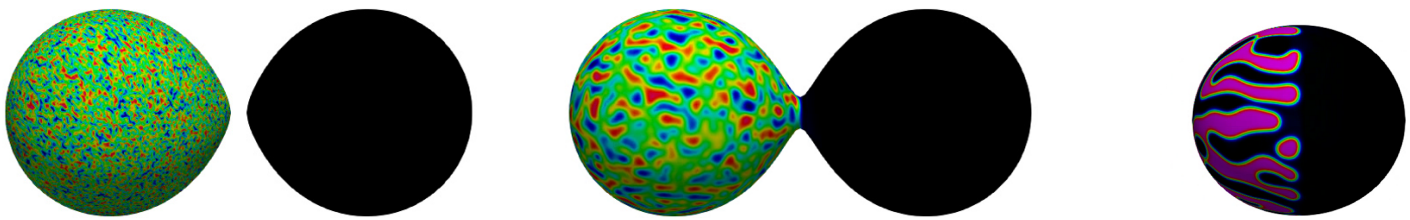
Slow collision

$t = 0.23$ $t = 0.26$ $t = 1.23$



Fast collision

$t = 0.023$ $t = 0.025$ $t = 0.123$



One paper reporting on these results has been published in in the Journal of Computational Physics in 2020.

Comparison with Majd's experiments

We compared the numerical results produced by our geometrically unfitted solver for the Cahn–Hilliard model on steady surfaces with experiments conducted in Majd's lad for two membrane compositions:

- DOPC:DPPC with a 3:1 molar ratio with 20% Cholesterol (referred to as 3:1:20%), yielding $9(\pm\ 1.1)\%$ lipid domain area fraction.
- DOPC:DPPC with a 2:1 molar ratio with 20% Cholesterol (referred to as 2:1:20%), yielding $15.7(\pm\ 1.5)\%$ lipid domain area fraction.

To set the initial state for numerical simulations, we relied on thermodynamic considerations.

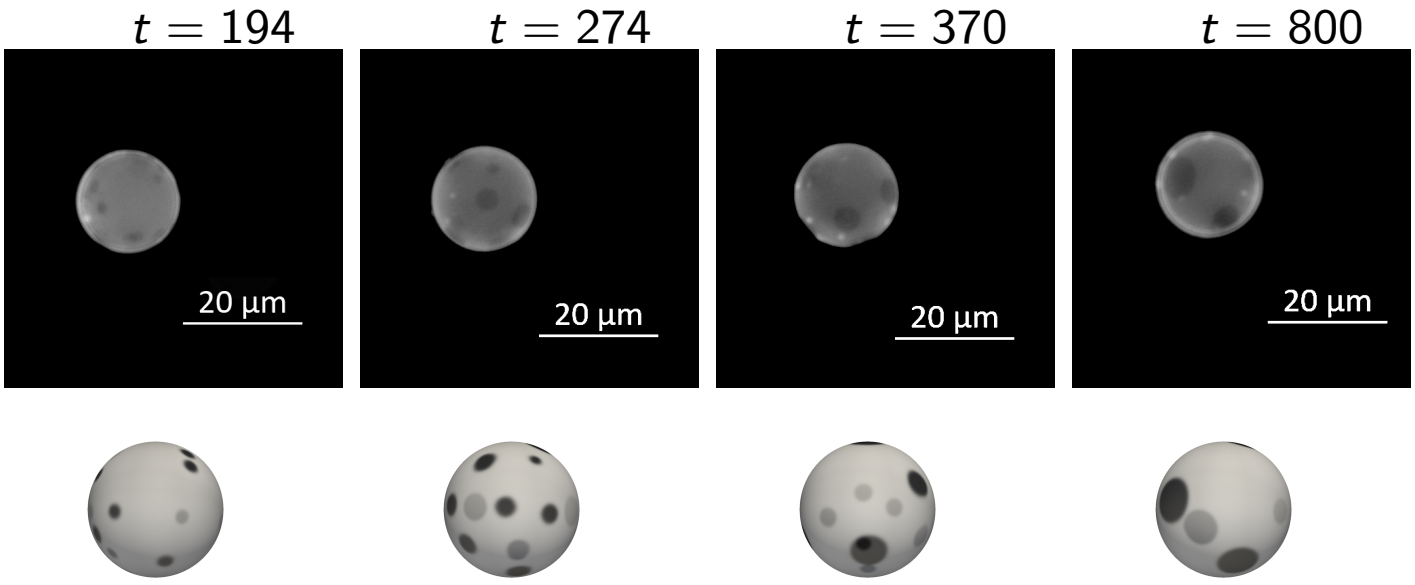
For both compositions, we performed:

- a qualitative comparison between numerical results and epi-fluorescence microscopy images.
- a quantitative comparison total lipid domain perimeter over time and total number of lipid domains over time.

The results are reported in a paper appeared in BBA - Biomembranes in 2021.

Qualitative comparison for composition 3:1:20%

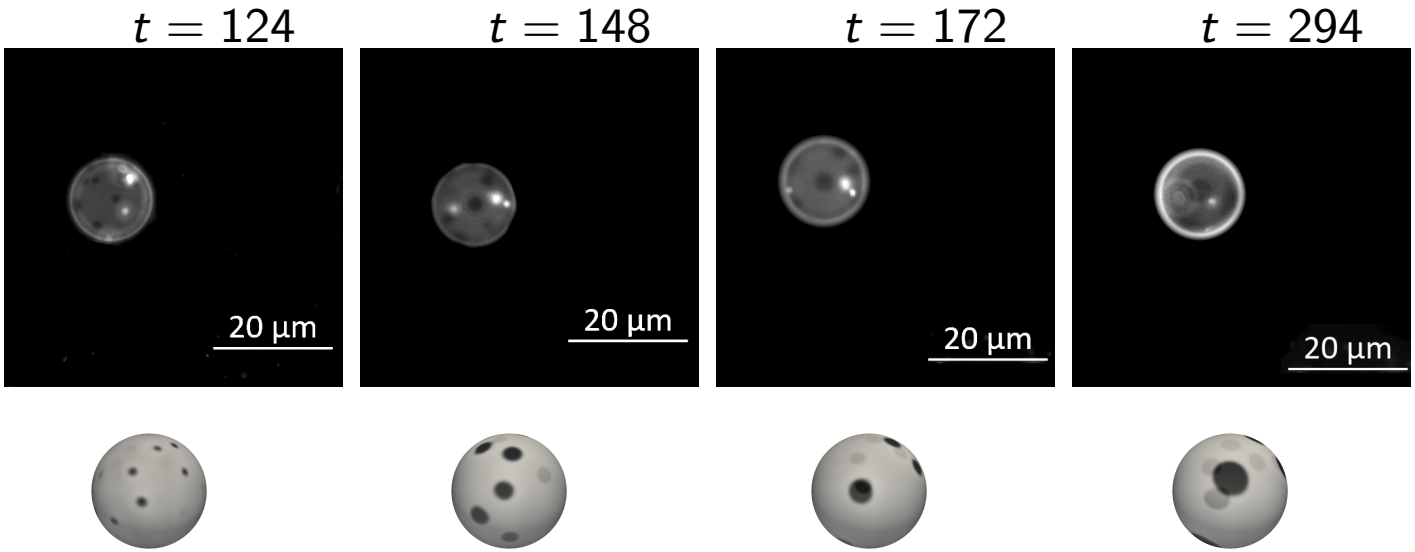
Epi-fluorescence microscopy images (with black background) and numerical results (with white background) at four different times in time interval $[194, 800]$ s.



Click any picture above to run the full animation of a representative simulation.

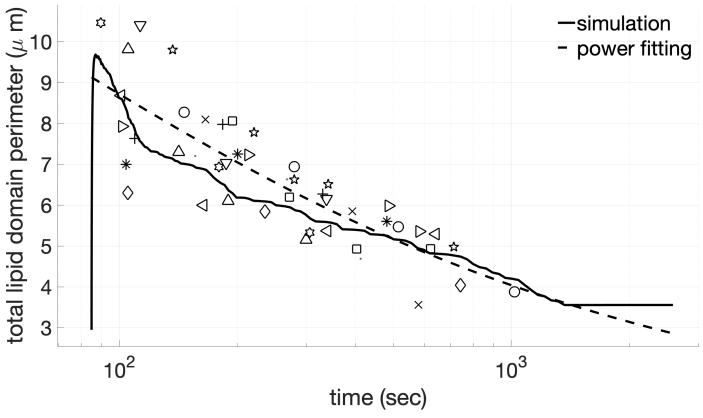
Qualitative comparison for composition 2:1:20%

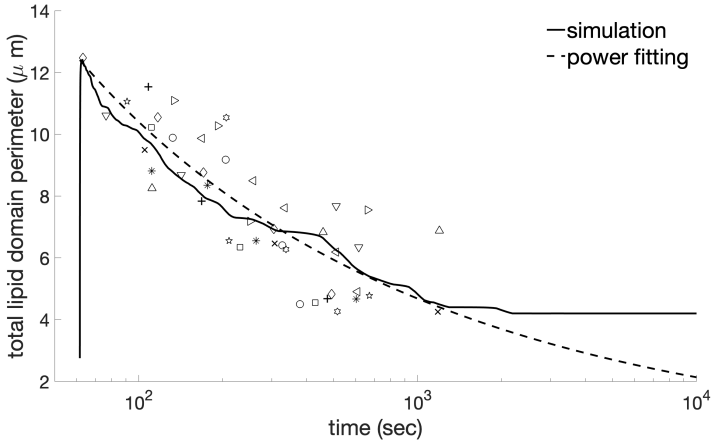
Epi-fluorescence microscopy images (with black background) and numerical results (with white background) at four different times in time interval $[124, 294]$ s



Click any picture above to run the full animation of a representative simulation.

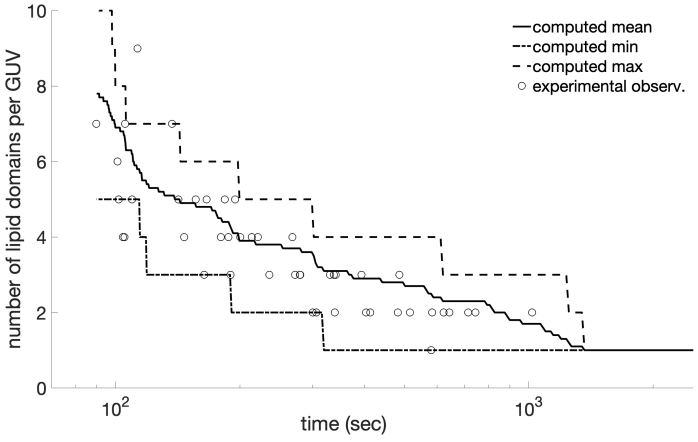
Quantitative comparison with Majd's experiments

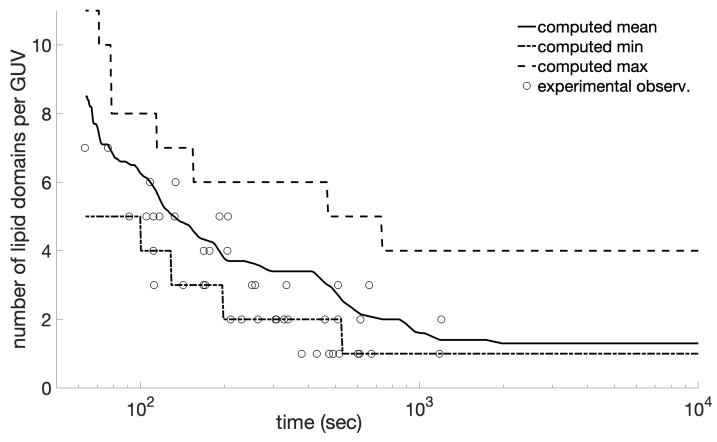




Left: Total lipid domain perimeter over time for composition 3:1:20%: numerical results average (solid line), power curve fitting (dashed line), experimental data (markers). Right: Total lipid domain perimeter over time for composition 2:1:20%: numerical results average (solid line), power curve fitting (dashed line), experimental data (markers).

Quantitative comparison with Majd's experiments





Total number of lipid domains over time for composition 3:1:20% (left) and 2:1:20% (right): numerical results average (solid line), minimal and maximum values found numerically (dash-dotted and dashed lines, respectively), and experimental data (circles).

In conclusion, our computational approach delivers not only qualitative pictures, but also accurate quantitative information about the dynamics of the membrane organization.

Navier–Stokes–Cahn–Hilliard system

While the results produced by the Cahn–Hilliard model agree well with the considered experimental data, they do not capture the viscous and fluidic phenomena present in lipid vesicles. Thus, we proposed a more complex thermodynamically consistent phase-field model.

$$\rho\partial_t\mathbf{u}+\rho(\nabla_\Gamma\mathbf{u})\mathbf{u}-\mathbf{P}\mathrm{div}_\Gamma(2\eta E_s(\mathbf{u}))+\nabla_\Gamma p=-\sigma_\gamma c\nabla_\Gamma\mu+M\theta(\nabla_\Gamma(\theta\mathbf{u}))\nabla_\Gamma\mu+\mathbf{f},$$

$$\mathrm{div}_\Gamma\mathbf{u}=0,$$

$$\partial_t c+\mathrm{div}_\Gamma(c\mathbf{u})-\mathrm{div}_\Gamma(M\nabla_\Gamma\mu)=0,$$

$$\mu=\tfrac{1}{\epsilon}f_0'-\epsilon\Delta_\Gamma c.$$

- η: dynamic viscosity of the mixture
- $\mathbf{u}$: surface averaged tangential velocity
- $E_s(\mathbf{u})$: surface rate-of-strain tensor
- σ_γ: line tension
- p: fluid pressure
- $\theta^2 = \frac{d\rho}{dc}$

Thanks to the term in red, the model allows for a non-linear dependence of fluid density on the phase-field order parameter.

Navier–Stokes–Cahn–Hilliard system

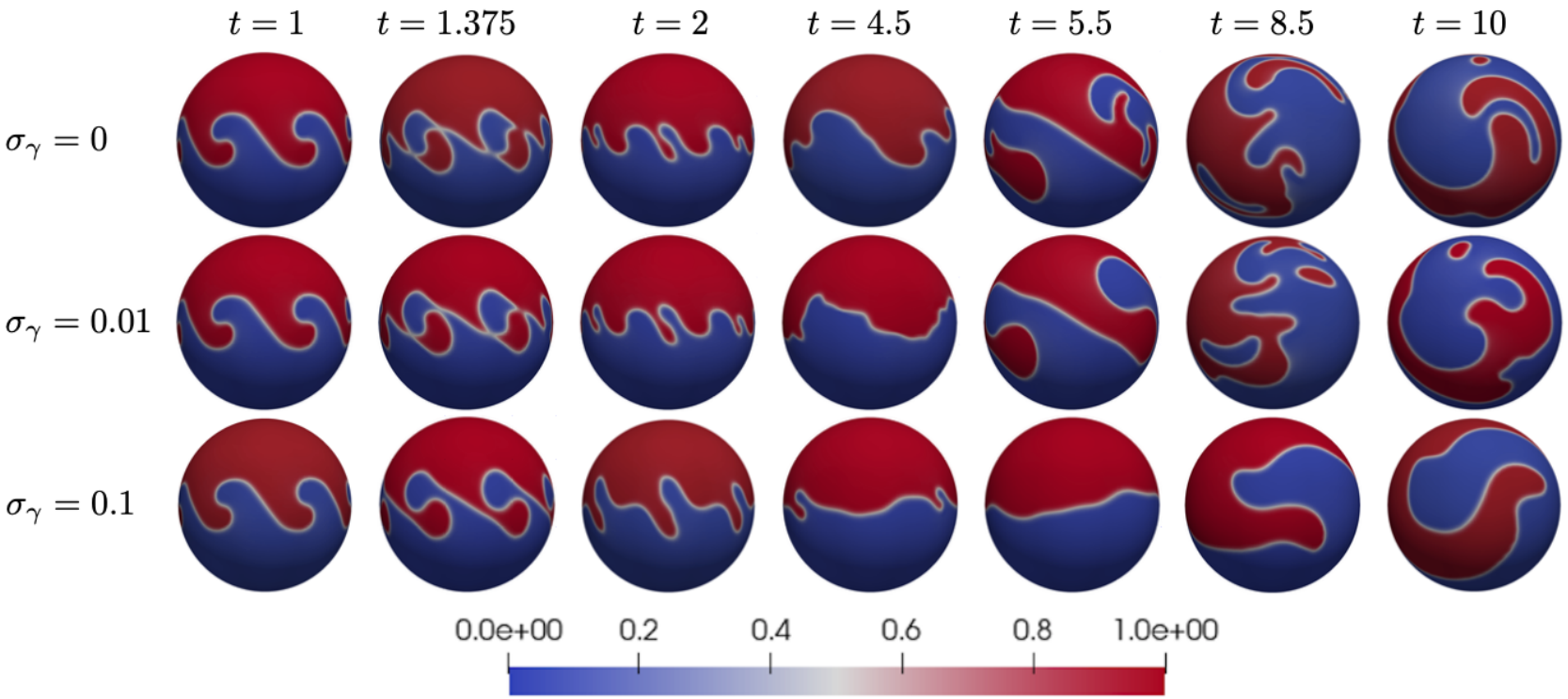
We apply an unfitted finite element method (TraceFEM) to discretize the system and introduce a fully discrete time-stepping scheme with the following properties:

1. the scheme decouples the fluid and phase-field equation solvers at each time step;
2. the resulting two algebraic systems are linear;
3. the numerical solution satisfies the same stability bound as the solution of the original system under some restrictions on the discretization parameters.

One paper on the Navier–Stokes–Cahn–Hilliard model and the proposed method has been submitted.

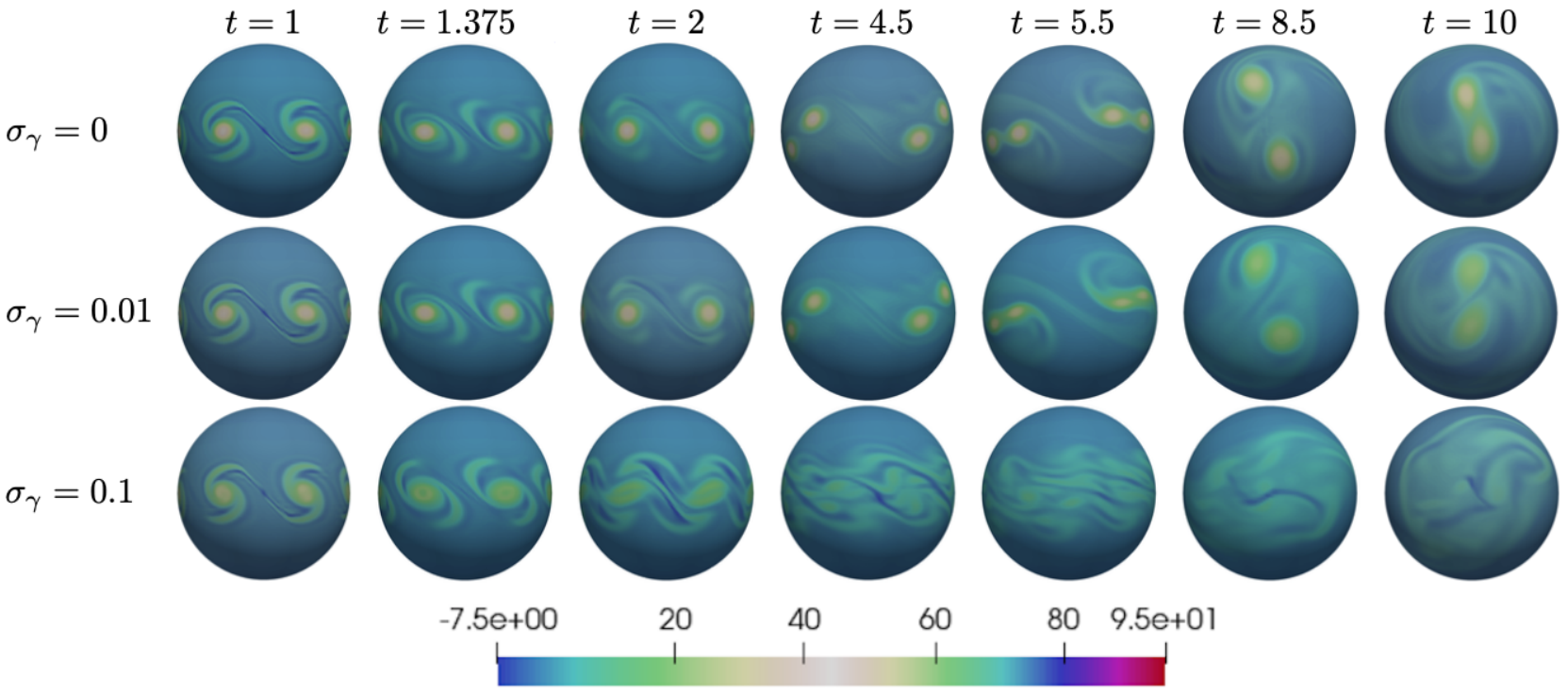
Kelvin–Helmholtz instability on a sphere

With the proposed method we could study the effect of line tension on the surface Kelvin–Helmholtz instability, a well-known two-phase fluid flow.



Evolution of order parameter for different values of line tension: $\sigma_\gamma = 0$ (top), $\sigma_\gamma = 0.01$ (center), and $\sigma_\gamma = 0.1$ (bottom). A full animation can be viewed following the link youtu.be/C3_WLO1Wd7Y

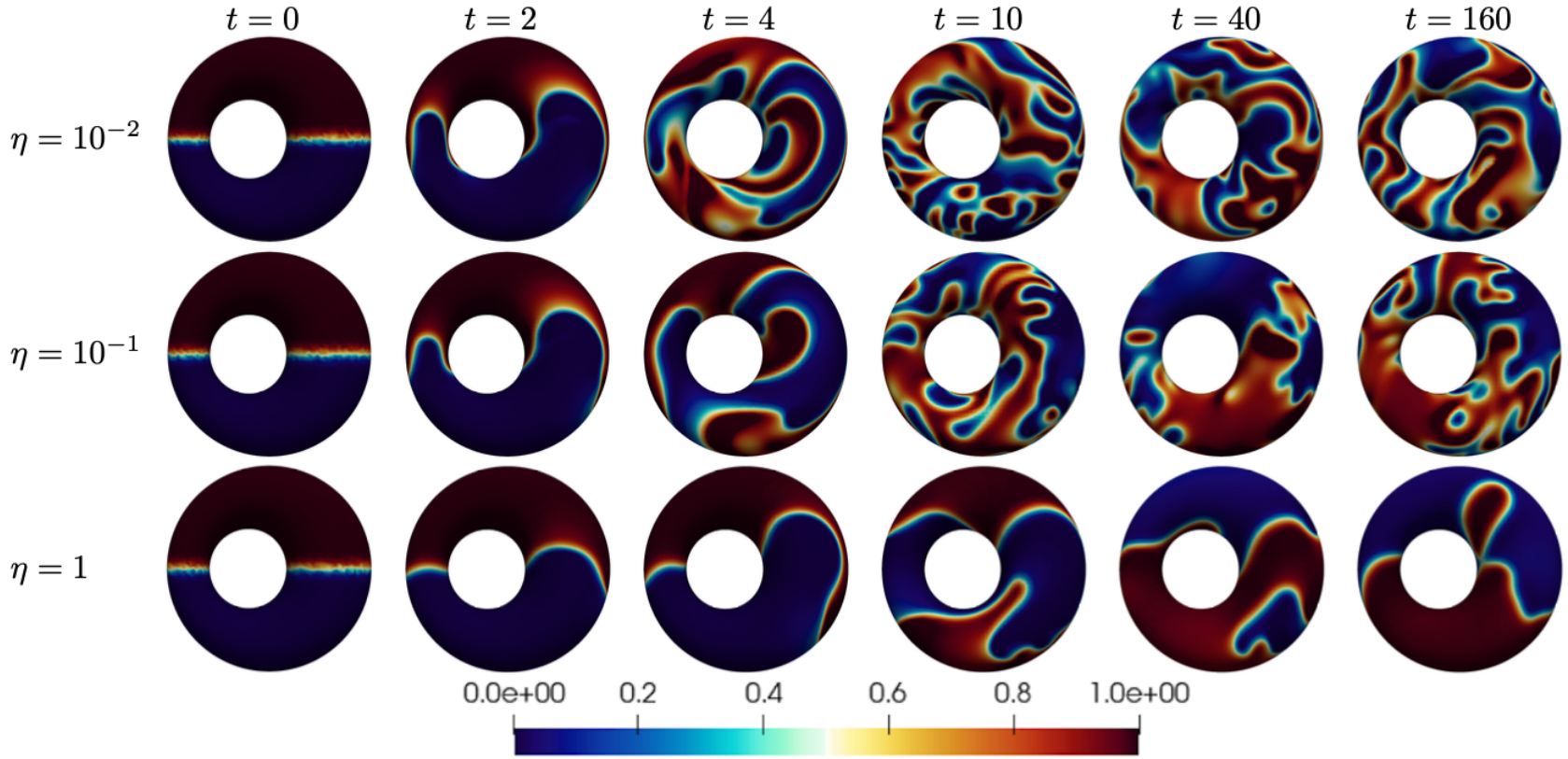
Kelvin–Helmholtz instability on a sphere



Evolution of the vorticity for different values of line tension: $\sigma_\gamma = 0$ (top), $\sigma_\gamma = 0.01$ (center), and $\sigma_\gamma = 0.1$ (bottom). A full animation can be viewed following the link youtu.be/FdMznBuMJPE

Rayleigh–Taylor instability on a torus

Another well-known two-phase fluid flow is the Rayleigh–Taylor instability. With the proposed method we could study the effect of viscosity and surface shape on the surface Rayleigh–Taylor instability.



Evolution of the order parameter for $\sigma_\gamma = 0.025$ and different values of viscosity: $\eta = 10^{-2}$ (top), $\eta = 10^{-1}$ (center), and $\eta = 1$ (bottom). A full animation can be viewed following the link youtu.be/FTqqFjvzEZg

Open-source library

All the computational results presented so far have been performed with open-source software DROPS:

`http://www.igpm.rwth-aachen.de/DROPS/`

An important outcome of this work is that the code created for it will be incorporated in DROPS as an open source resource available to the scientific and the engineering community.

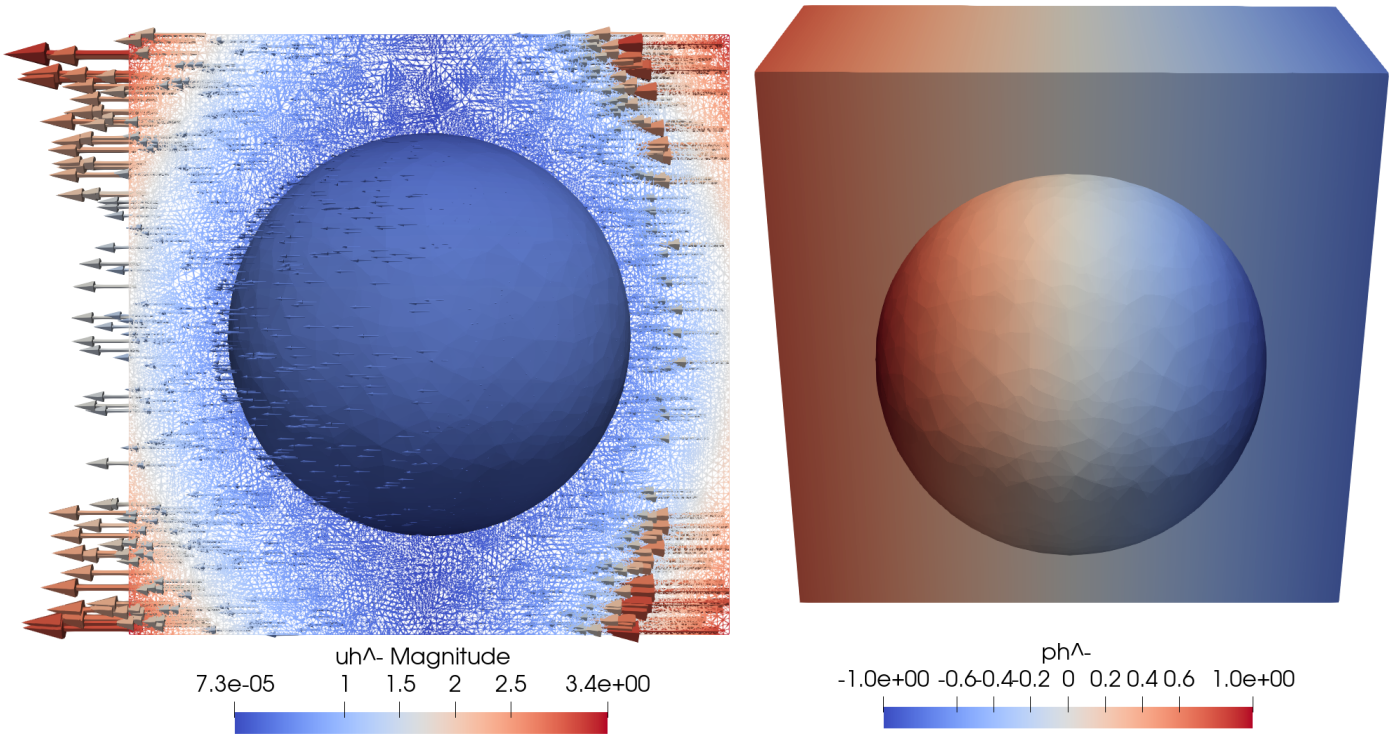
Two-phase Stokes problems with slip between phases

Liposomes encapsulate drugs and are injected into the blood stream to reach their target. Thus, liposomes interact with fluid around and within them. As a first step towards modeling this complex interaction, we worked on two-phase Stokes problems with slip between phases. The two phases would represent the fluid inside and outside the liposome, which have different physical parameters.
For the two-phase Stokes problems we proposed an isoparametric unfitted finite element approach of the CutFEM or Nitsche-XFEM family. In particular, for a class of unfitted finite elements

- we showed an inf-sup stability property with a stability constant that is independent of the viscosity ratio, slip coefficient, position of the interface with respect to the background mesh and, of course, mesh size;
- we proved stability and optimal error estimates that follow from this inf-sup property;
- we provided numerical results in two and three dimensions to corroborate the theoretical findings and demonstrated the robustness of our approach.

3D two-phase Stokes problem with exact solution

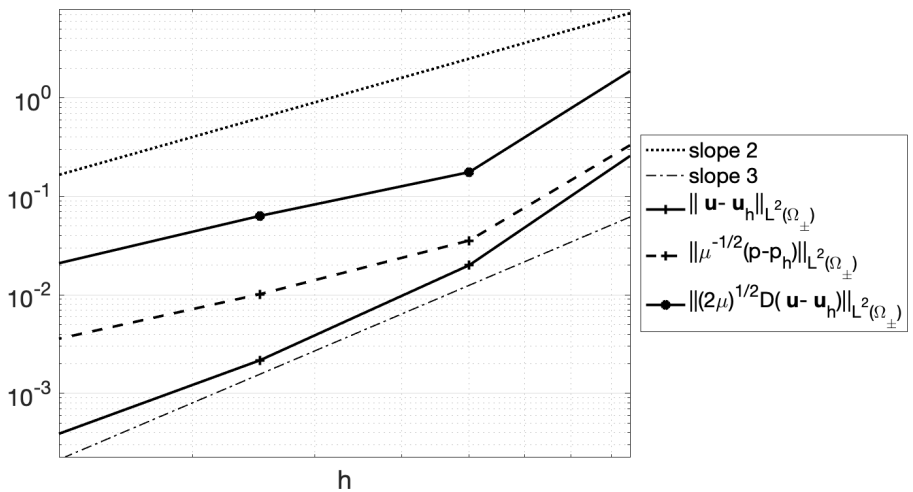
We considered a spherical interface between the two phases and the exact solution depicted in the figure below. Let Ω^+ be the domain outside the sphere and Ω^- the domain inside the sphere.



Velocity vectors colored with the velocity magnitude on the *xz*-section of Ω^+ and in Ω^- (left) and pressure in Ω^- and half Ω^+ (right).

3D two-phase Stokes problem with exact solution

We considered structured meshes of tetrahedra with four levels of refinement. The initial triangulation has mesh size $h = 1$ and all the other meshes are obtained by halving h till $h = 0.125$. We chose to use finite element pair $\mathbf{P}_2 - P_1$. Below, we show the L^2 error and a weighted H^1 error for the velocity and a weighted L^2 error for the pressure against the mesh size h.

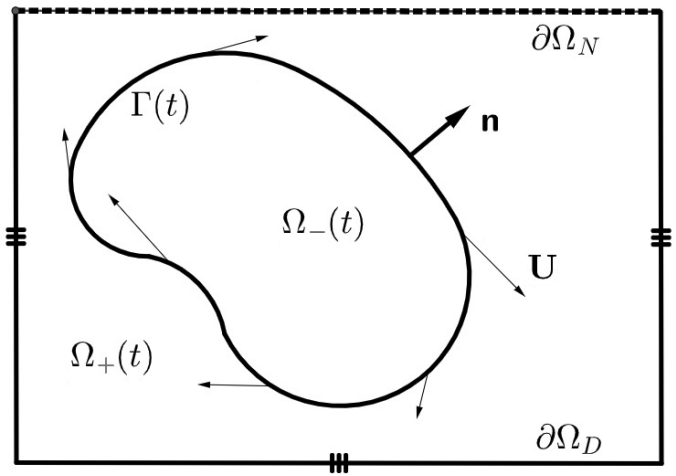


We observe almost cubic convergence in the L^2 norm for the velocity, quadratic convergence in the weighted L^2 norm for the pressure and in the weighted H^1 norm for the velocity.

One paper with these results has been submitted.

Coupling bulk and surface fluid flows

As a starting point, we considered a simplified version the coupled bulk and surface flow problem: we assumed the system has reached a steady state and inertia terms can be neglected.



Bulk flow in $\Omega_\pm$:

$$-\mu^\pm \Delta \mathbf{u}^\pm + \nabla p = \mathbf{f}^\pm,$$
$$\nabla \cdot \mathbf{u}^\pm = 0.$$

Surface flow on Γ:

$$-2\mu_\Gamma \mathbf{P}\mathrm{div}_\Gamma \mathbf{D}_\Gamma(\mathbf{U}_T) + \nabla_\Gamma \pi = [\mathbf{P}\boldsymbol{\sigma}\mathbf{n}]^+_-,$$
$$\mathrm{div}_\Gamma \mathbf{U}_T = 0.$$

Coupling conditions

The bulk and surface fluid are coupled by

- the tangential load exerted by the bulk fluid onto the surface fluid (see previous slide);
- balance of the normal component of the normal stresses:

$$[\mathbf{n}^T \boldsymbol{\sigma} \mathbf{n}]_+^- = \pi\kappa \qquad \text{on } \Gamma;$$

- the immiscibility condition:

$$\mathbf{u}^+ \cdot \mathbf{n} = \mathbf{u}^- \cdot \mathbf{n} \qquad \text{on } \Gamma;$$

- slip with friction between the bulk fluid and the viscous membrane:

$$\begin{aligned} \mathbf{P}\boldsymbol{\sigma}^+\mathbf{n} &= f^+(\mathbf{P}\mathbf{u}^+ - \mathbf{U}_T) && \text{on } \Gamma, \\ \mathbf{P}\boldsymbol{\sigma}^-\mathbf{n} &= -f^-(\mathbf{P}\mathbf{u}^- - \mathbf{U}_T) && \text{on } \Gamma. \end{aligned}$$

Coupling bulk and surface fluid flows

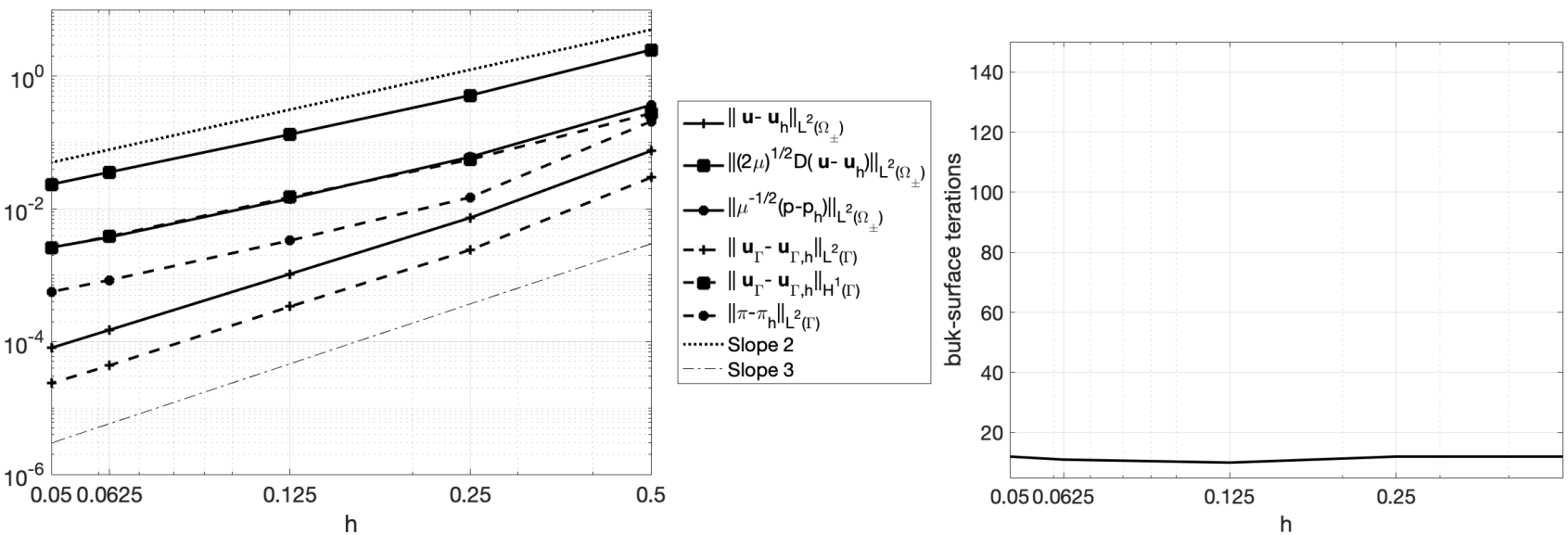
For this coupled problem:

- We proved well-posedness.
- We proposed a partitioned strategy for its numerical solution, i.e. bulk and surface flows are solved separately and the coupling conditions enforced in an iterative fashion. The closest domain decomposition approach to the decoupling scheme we proposed is the Robin-Neumann method.
- We provided numerical results in three dimensions to demonstrated the robustness of our approach.

One paper on this work is about to be completed.

Exact solution for the coupled problem

We considered an exact solution for a spherical interface between the two phases. To check the spatial accuracy of our scheme, we used structured meshes of tetrahedra with five levels of refinement, the coarsest mesh having mesh size $h = 0.5$ while the finest mesh has $h = 0.05$. We chose finite element pair $\mathbf{P}_2 - P_1$ for both the bulk and surface fluid problems. Below we report the convergence test (left) and the number of bulk-surface iterations of the partitioned method as h varies (right).



We observe optimal convergence rates for all the norms under consideration. Moreover, the number of iterations is fairly insensitive to a mesh refinement or coarsening.

Open-source library

All the computational results for the two-phase Stokes flow and coupled bulk-surface flow have been obtained with NGsolve:

`https://ngsolve.org/`

a high performance multiphysics finite element software with a Python interface, and add-on library ngsxfem[1], which enables the use of unfitted finite element technologies.

Just like in the case of DROPS, we will incorporate the code created for this project into ngsxfem as a way to give back to the scientific community.

[1] `https://github.com/ngsxfem/ngsxfem/tree/49205a1ae637771a0ed56d4993ce99008f3a00e0`

Plans for the Next Reporting Period

- Qualitative and quantitative comparison of the results produced by the Navier–Stokes–Cahn–Hilliard model with another series of experiments performed in Majd's lab.
- Development of a mathematical model and numerical methods to simulate liposome-membrane interaction driven by electrostatic forces.
- Deployment of the solver created for the previous point to quantify the uncertainty in the simulations and estimate the likelihood of a liposome with a given composition to fuse with a cell membrane.