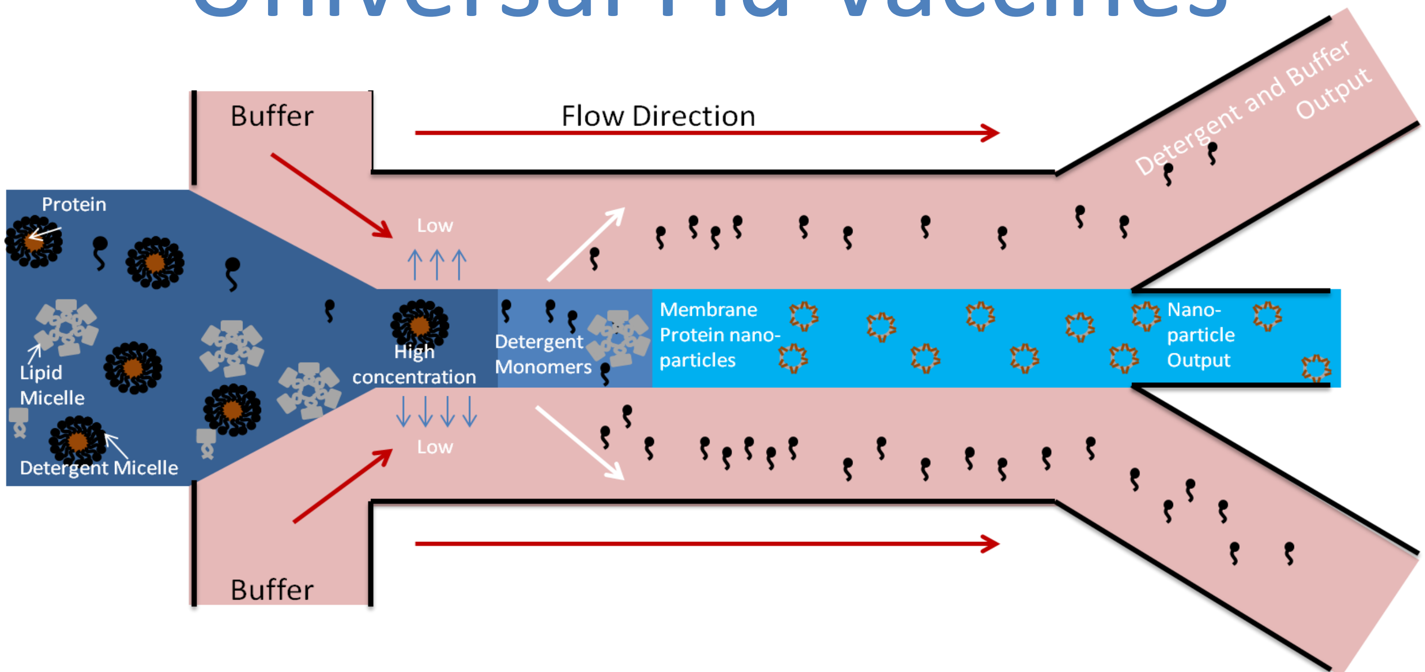
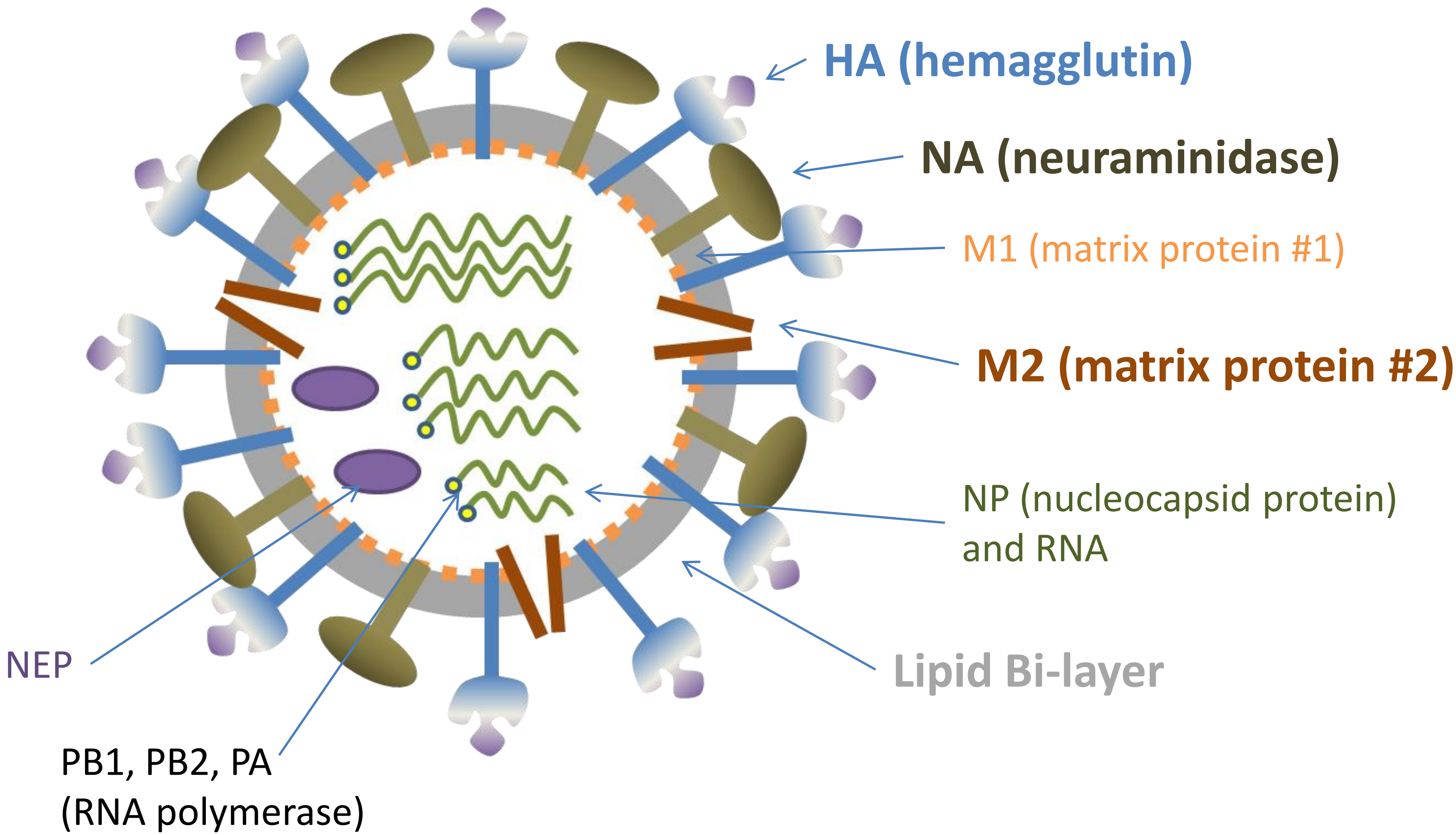
Bio/Nano/Micro-Engineering Universal Flu Vaccines

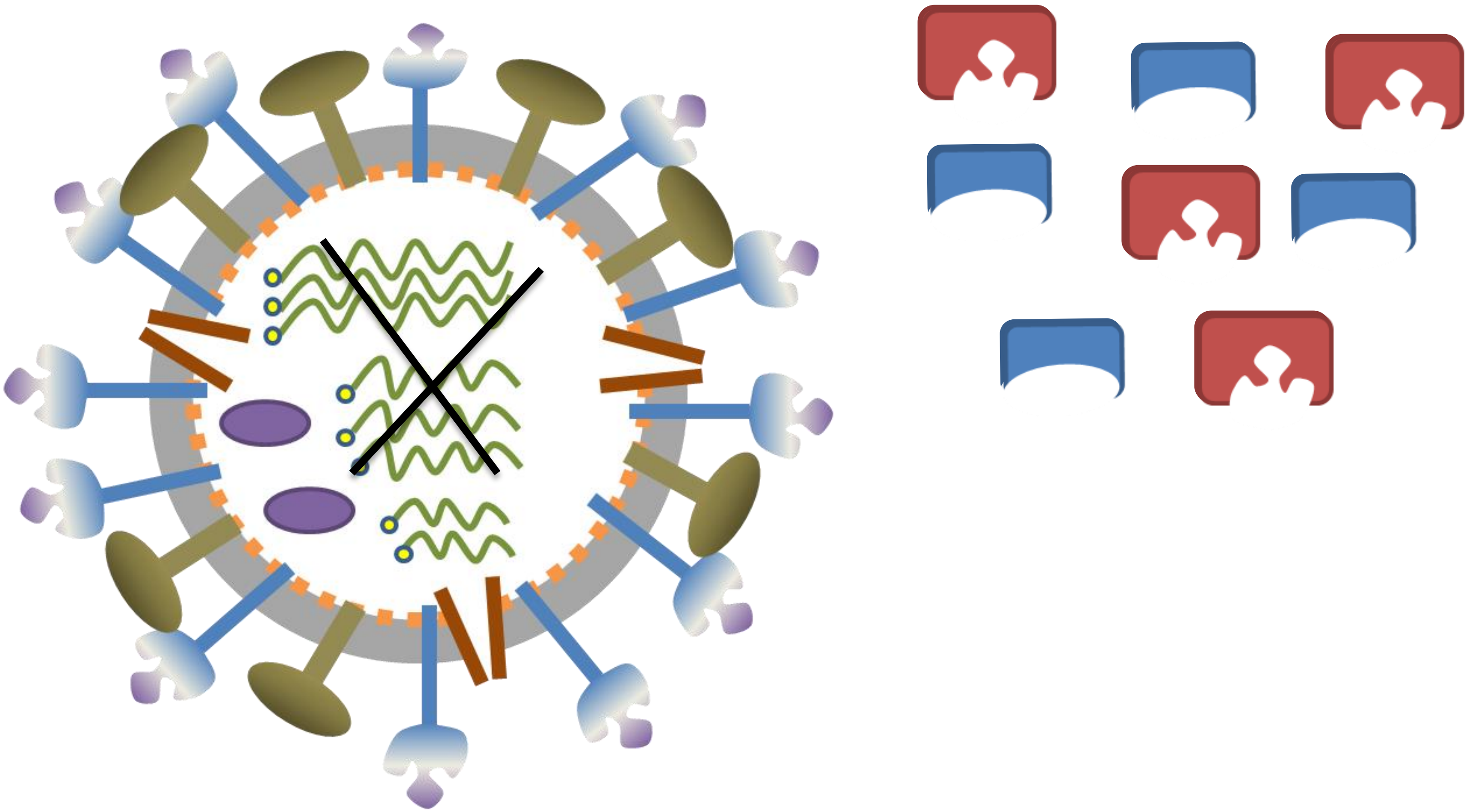


Ryan Lewis, Ph.D.
Dept of Mechanical Engineering
University of Colorado at Boulder
rjlewis@colorado.edu
Built on work by Ray Wu, Ph.D
hsin-jui.wu@colorado.edu

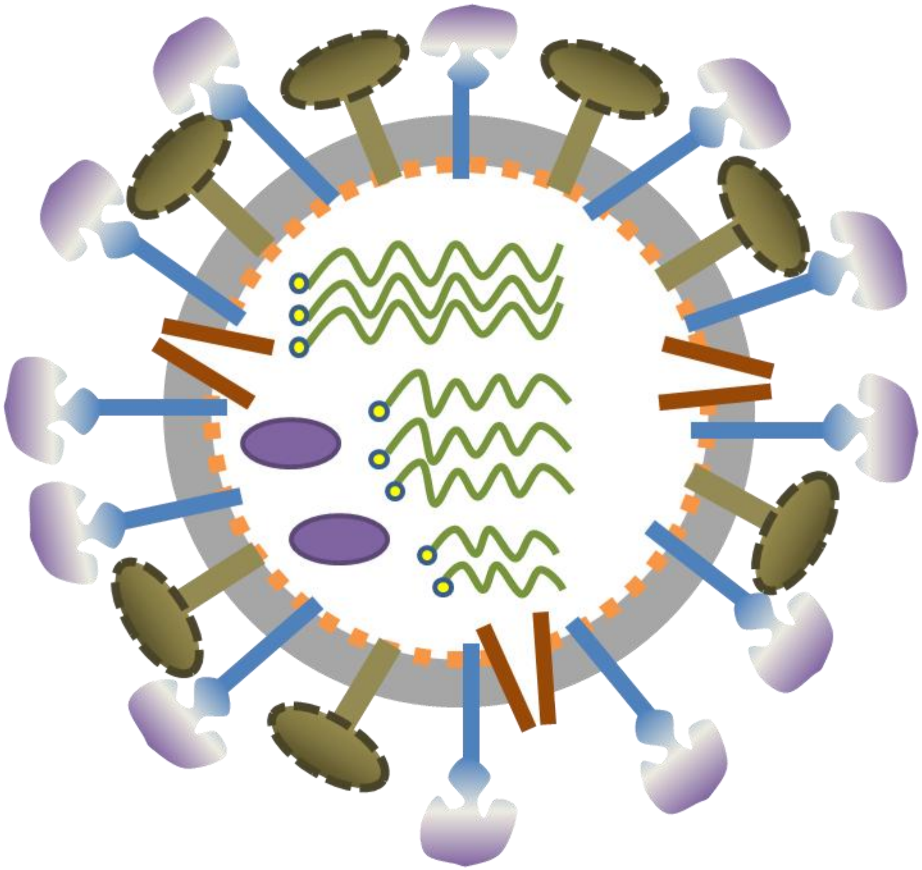
Flu Virus

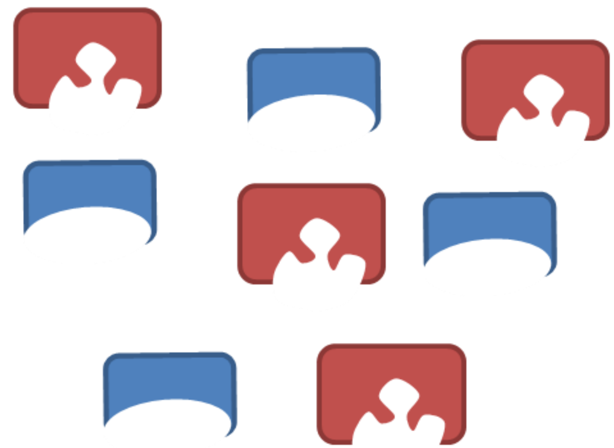

HA (hemagglutin)
NA (neuraminidase)
M1 (matrix protein #1)
M2 (matrix protein #2)
NP (nucleocapsid protein)
and RNA
Lipid Bi-layer
NEP
PB1, PB2, PA
(RNA polymerase)

Flu Vaccine



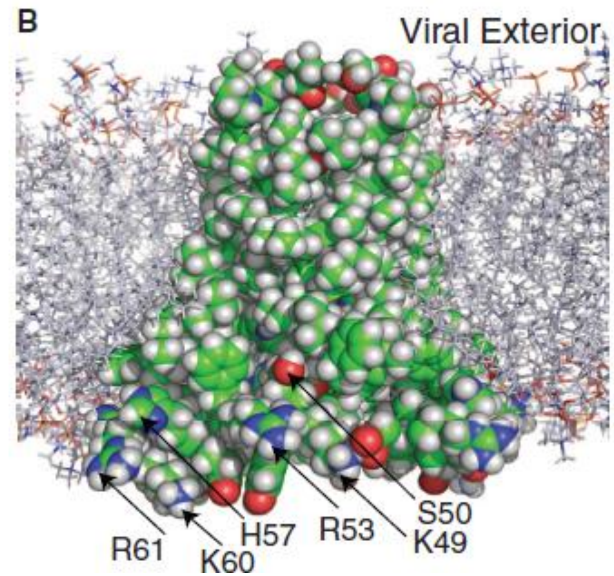
Flu Virus

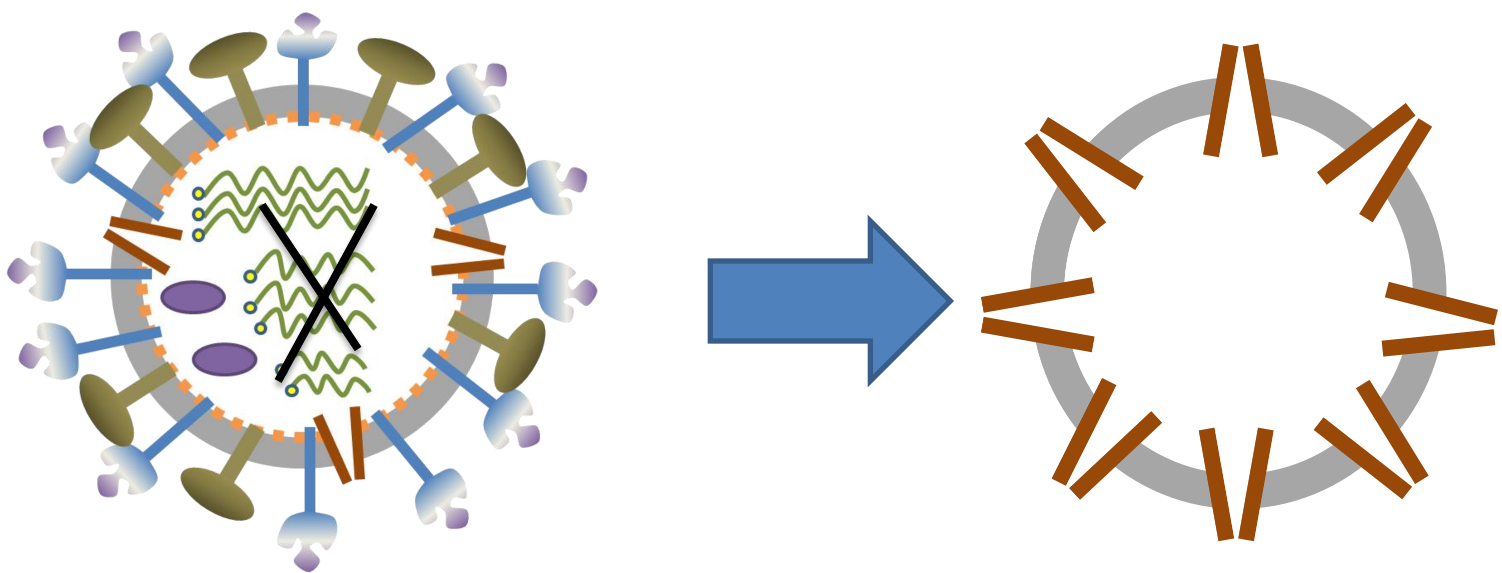




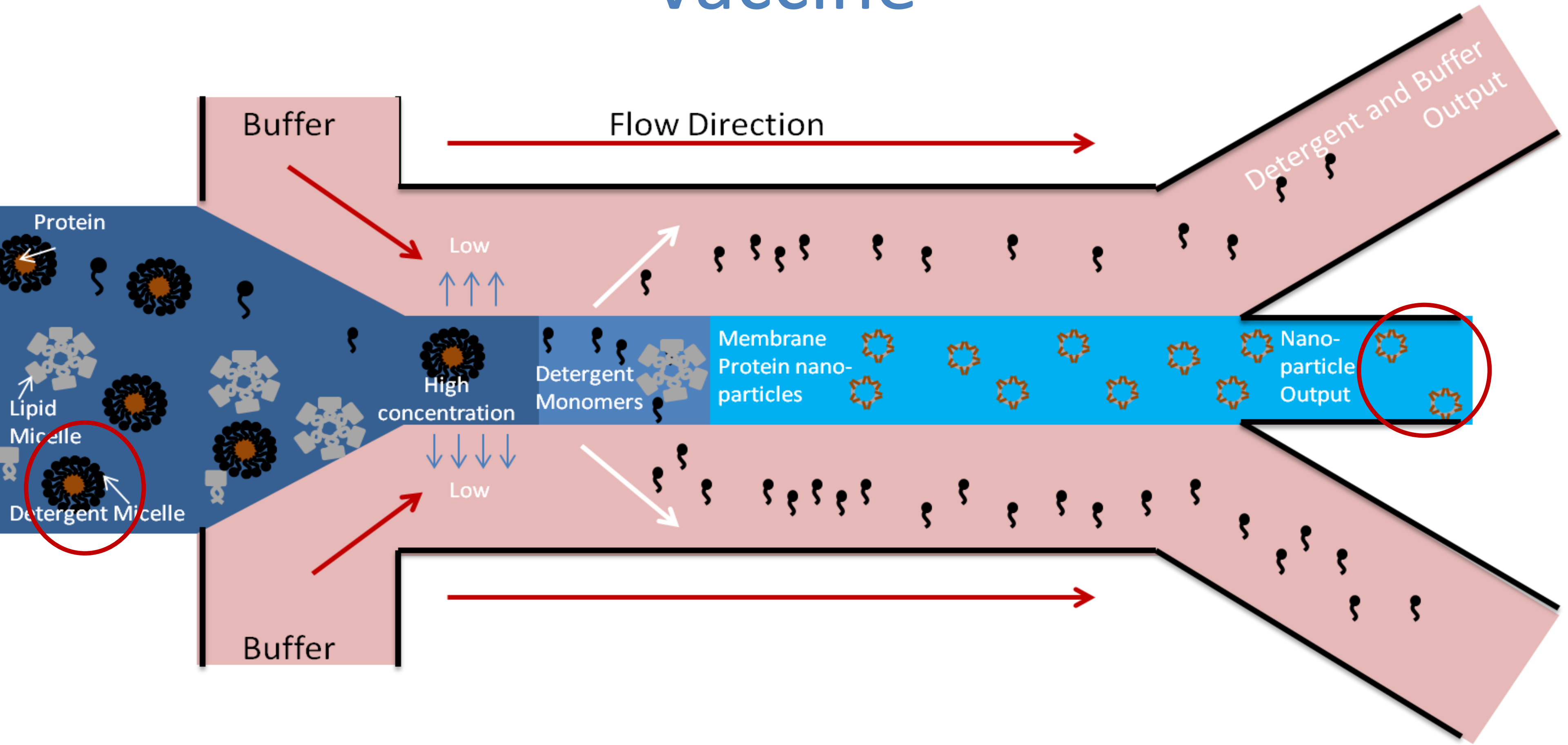
Universal Vaccine

- M2 Protein: conserved between types
- Membrane-protein polyhedral Nano-particle

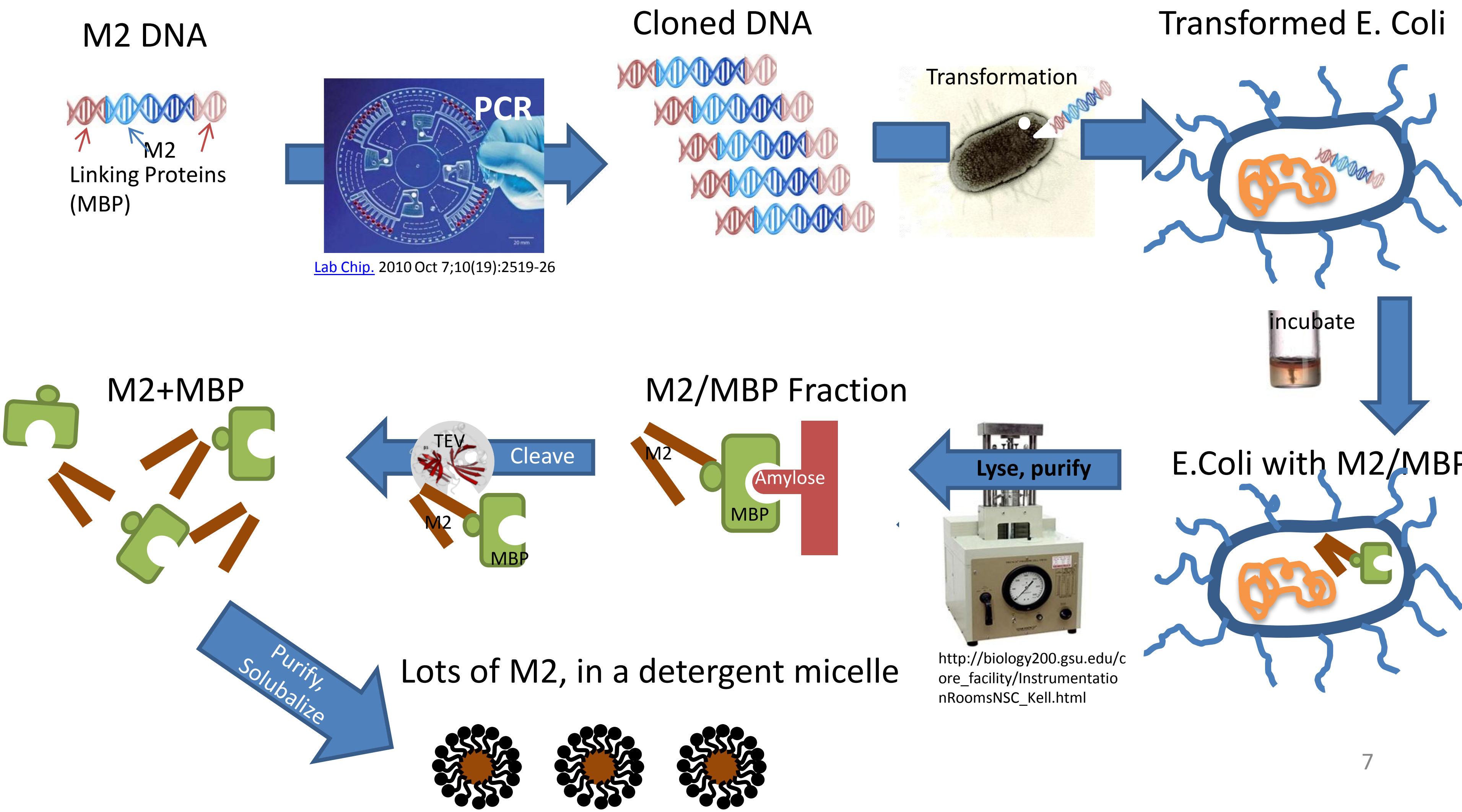




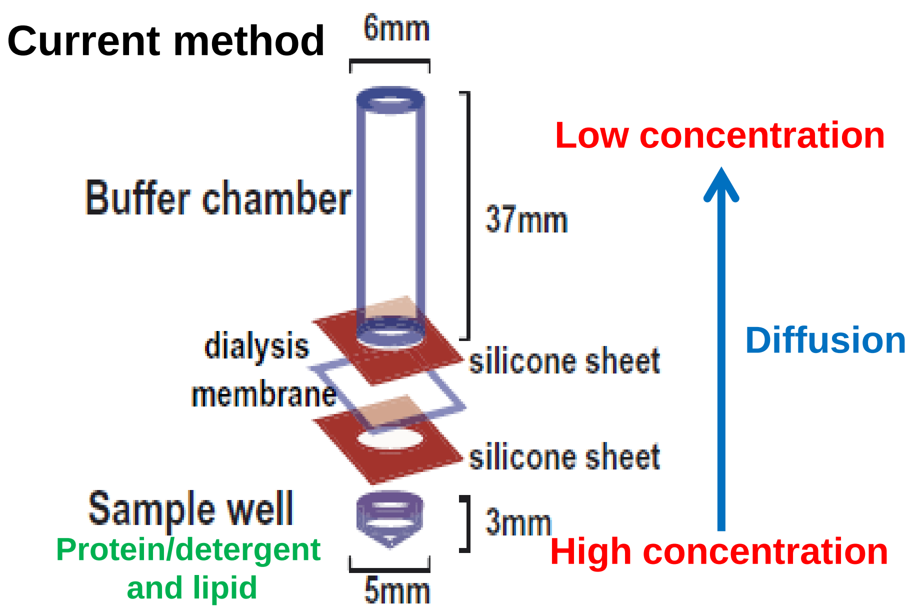
Bio/Micro Engineered Universal Flu Vaccine



Manufacturing M2



M2 Virus-Like Particles



M. Vink et al. Journal of Structural Biology 160, **2007**,295–304

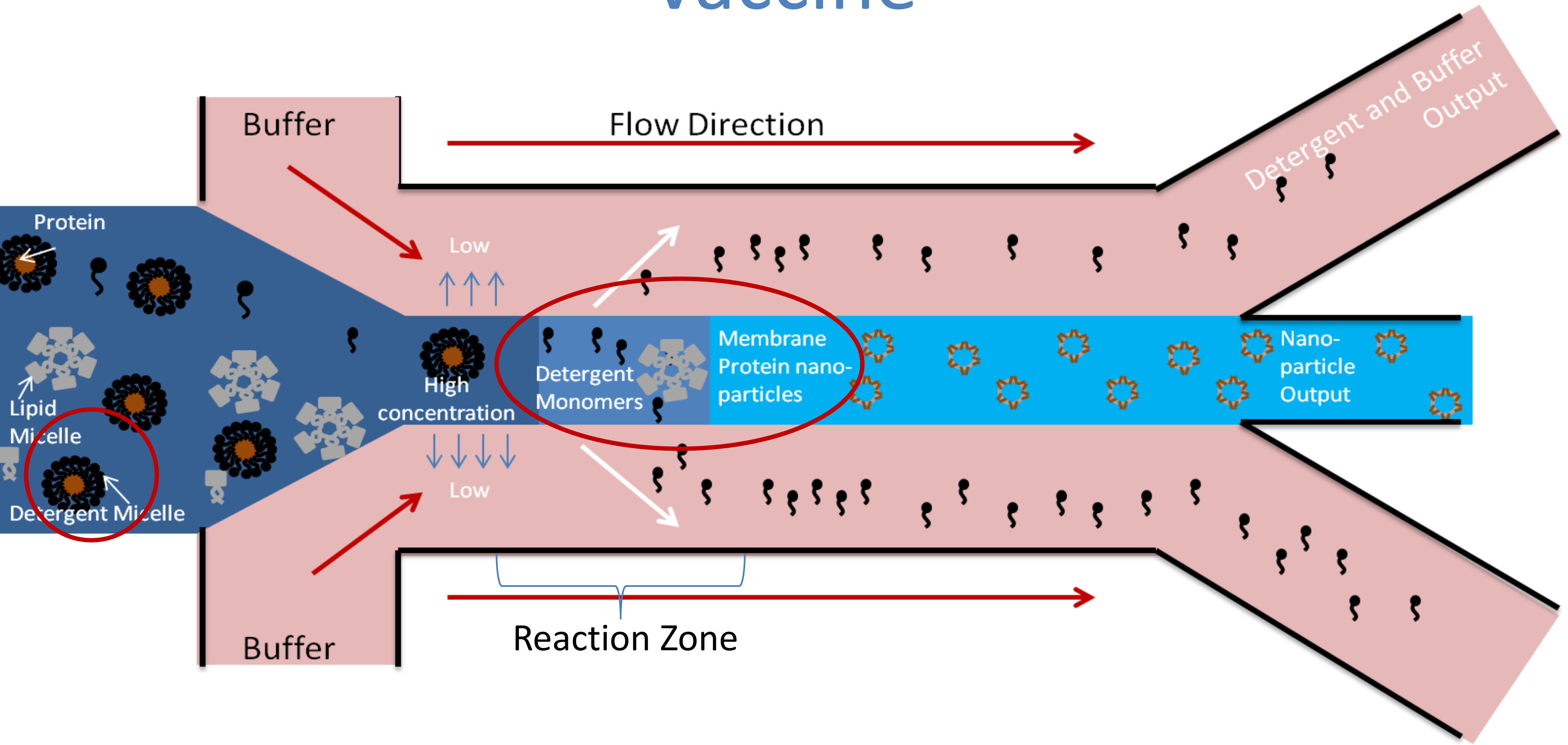
To get protein nano-particles, need:

- Right protein: Lipid ratio
- Right pH
- Right buffer concentration
- Right buffer solution
- "Right dandruff"

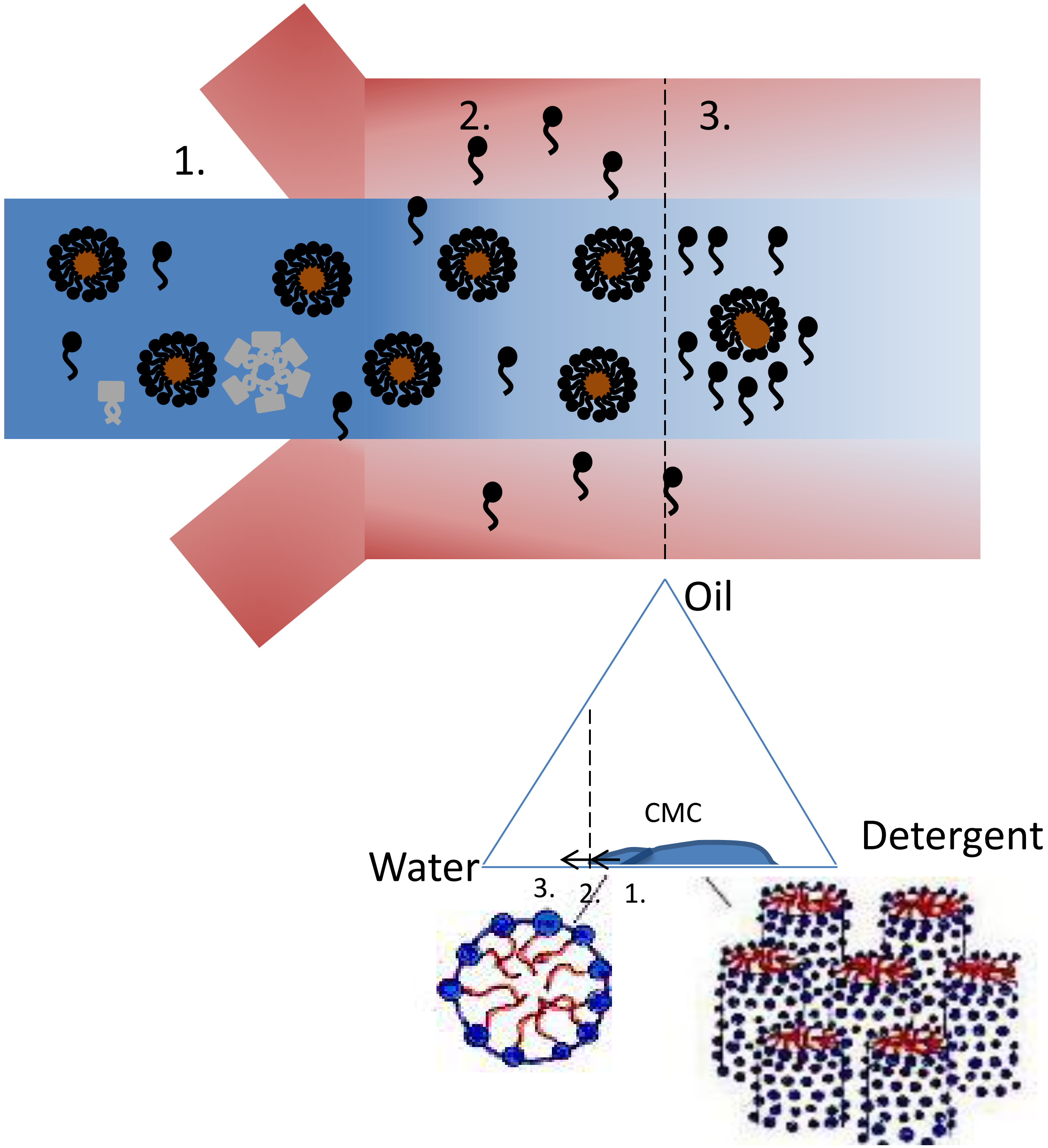
With all correct conditions

- Week-long reaction time
  - (mm diffusion length)
- µL or mL sample, per test

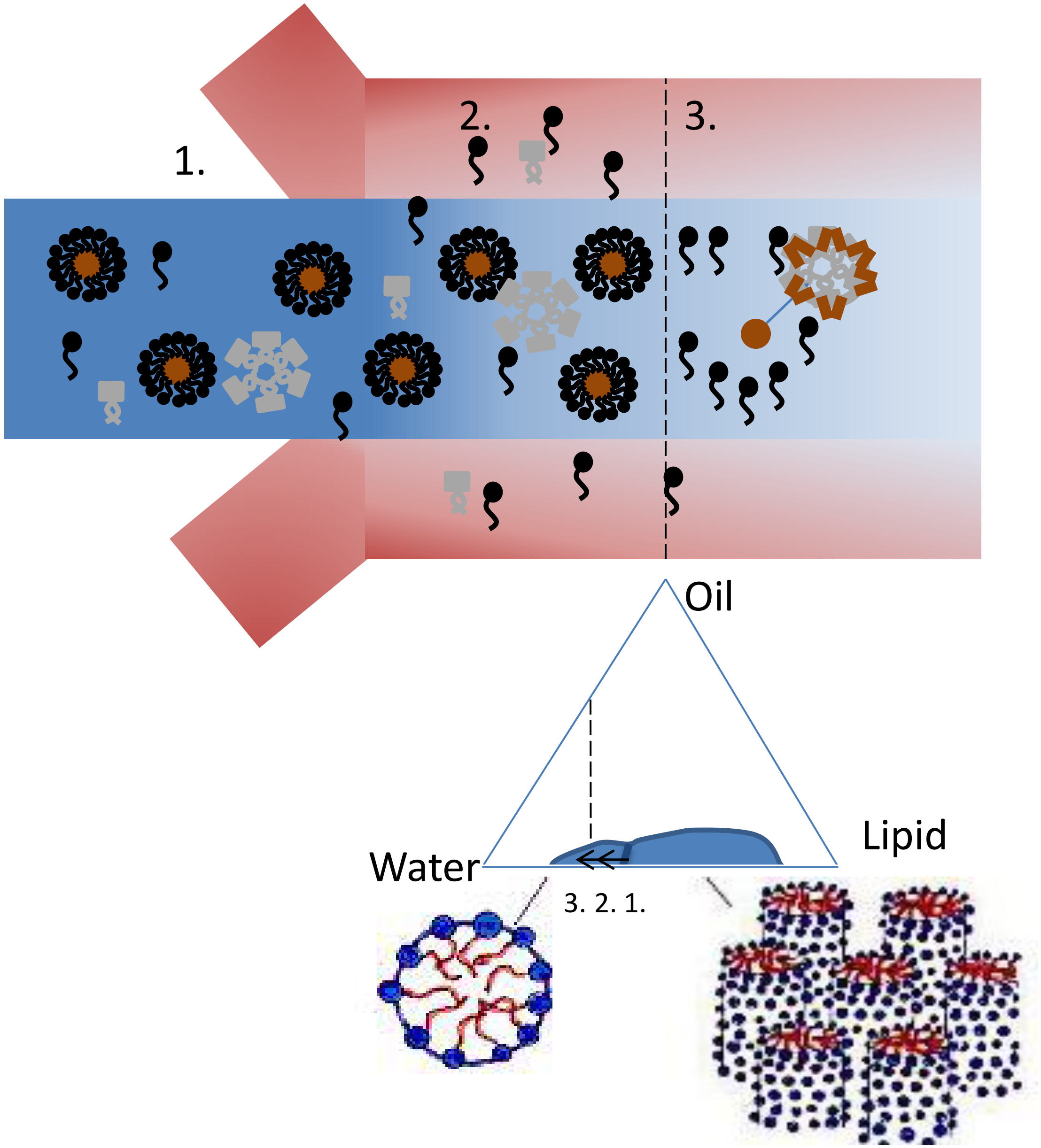
Bio/Micro Engineered Universal Flu Vaccine



Micro Fluid Reaction--Detergent

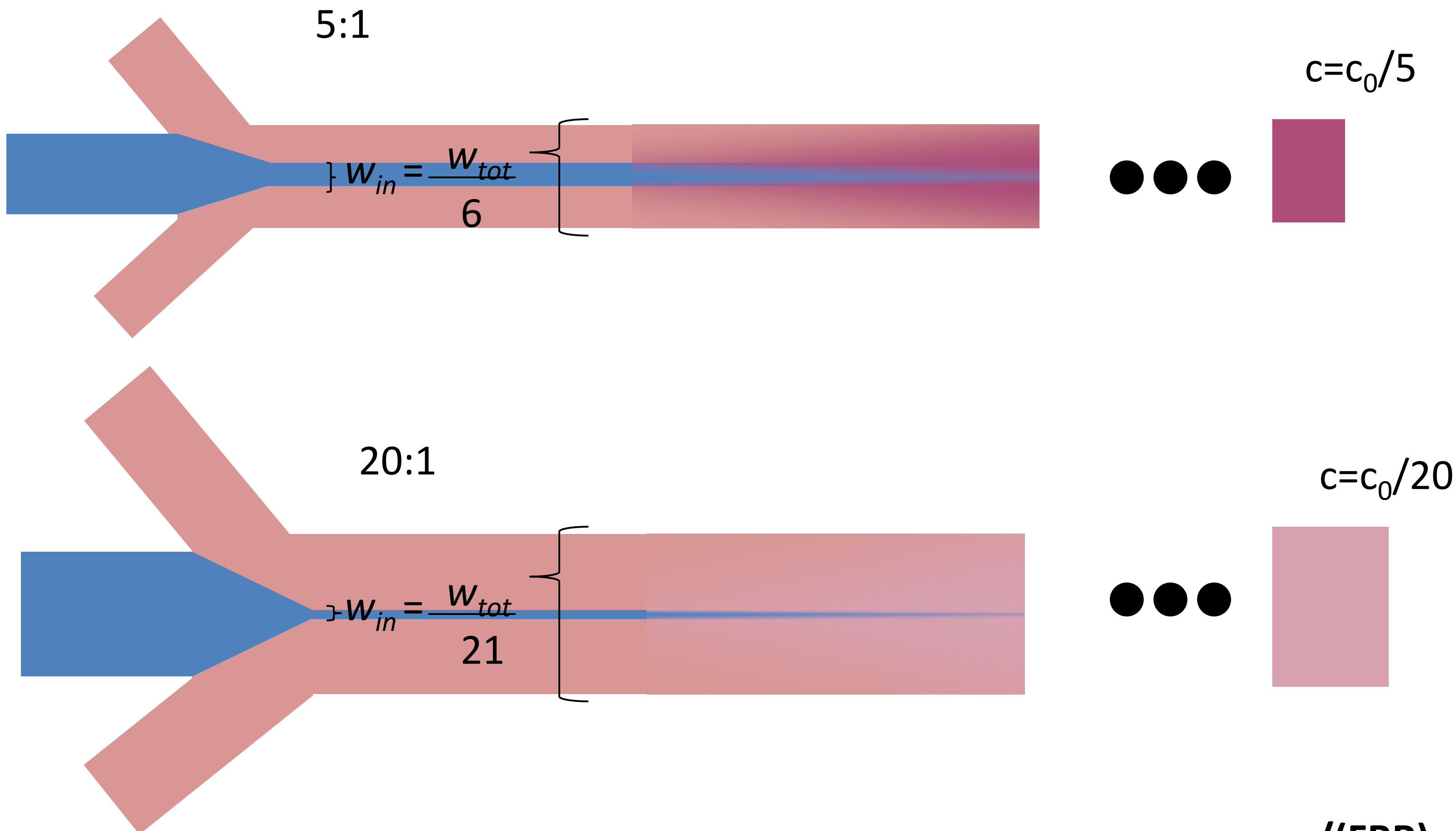


Micro Fluid Reaction--Lipid



Micro Fluid Reactor Design

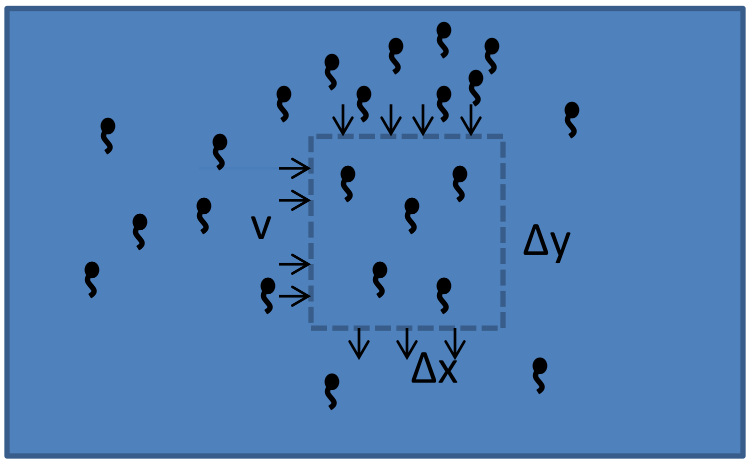
- Flow-Rate Ratio



$c_\infty=c_0/(FRR)$

Micro Fluid Reactor Design

- Convective Diffusion



$$\Delta z \Delta x \Delta y \frac{\partial c}{\partial t} =$$ Total mass influx from diffusion + Total mass influx from convection

Total mass influx from diffusion = Mass gain on top - Mass loss on bottom

$$\mathcal{D}\frac{\partial c}{\partial y}\Big|_{top} \Delta x \Delta z - \mathcal{D}\frac{\partial c}{\partial y}\Big|_{bottom} \Delta x \Delta z$$

Total mass influx from convection = Mass gain in left - Mass loss out right

$$\left(vc_{left} - vc_{right}\right)\Delta y \Delta z$$

$$\frac{\partial c}{\partial t} = \frac{\mathcal{D}\frac{\partial c}{\partial y}\Big|_{top} - \mathcal{D}\frac{\partial c}{\partial y}\Big|_{bottom}}{\Delta y} + \frac{\left(vc_{left} - vc_{right}\right)}{\Delta x} = \boxed{\mathcal{D}\frac{\partial^2 c}{\partial^2 y} - v\frac{\partial c}{\partial x} = 0}$$

Micro Fluid Reactor Design

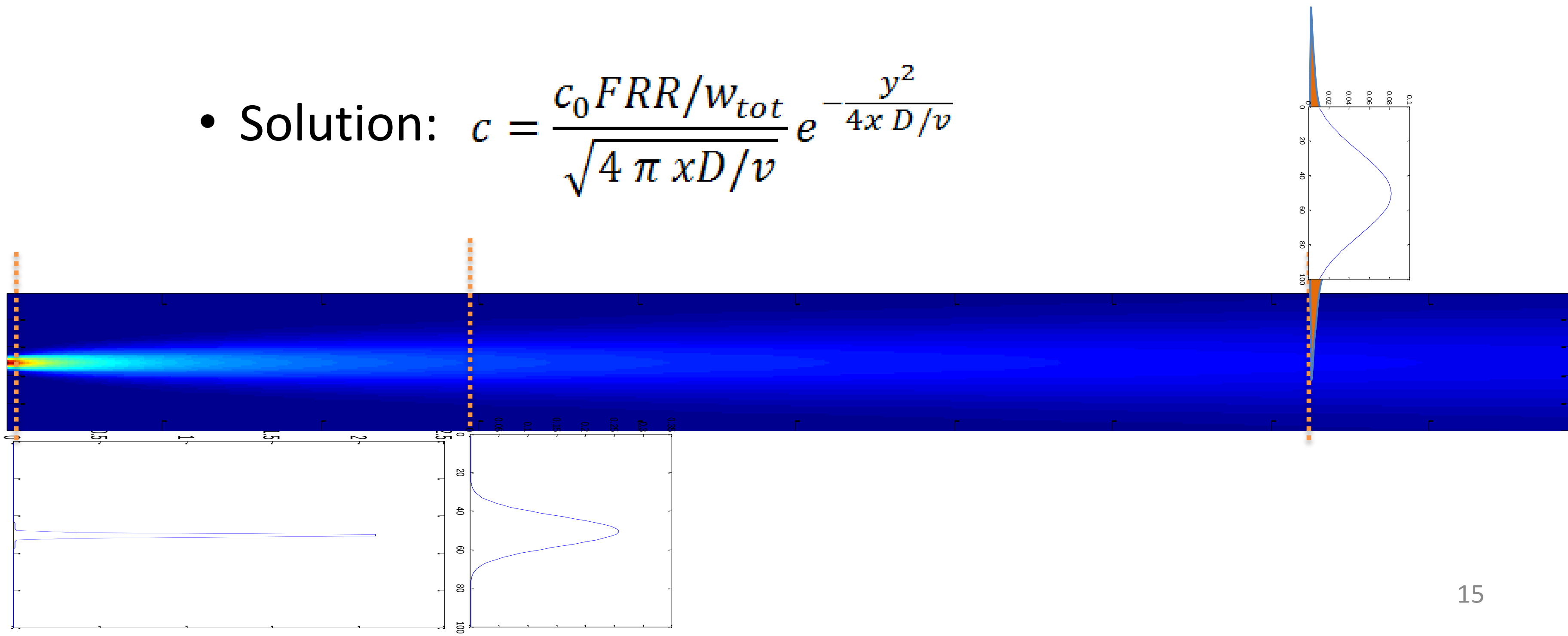
- Convective Diffusion $0 = \mathcal{D}\frac{\partial^2 c}{\partial^2 y} - v\frac{\partial c}{\partial x}$
  - More accurate:

$$\left\{\begin{aligned} \frac{\partial c}{\partial t} &= \mathcal{D}\nabla^2 c + \vec{\boldsymbol{v}}(x,y)\cdot\vec{\boldsymbol{\nabla}}c \\ \rho(\vec{\boldsymbol{v}}\cdot\boldsymbol{\nabla})\vec{\boldsymbol{v}} &= -\boldsymbol{\nabla}P + \mu\nabla^2\vec{\boldsymbol{v}} \\ \nabla\cdot\vec{\boldsymbol{v}} &= 0 \end{aligned}\right\}$$

Need numeric solutions

Micro Fluid Reactor Design

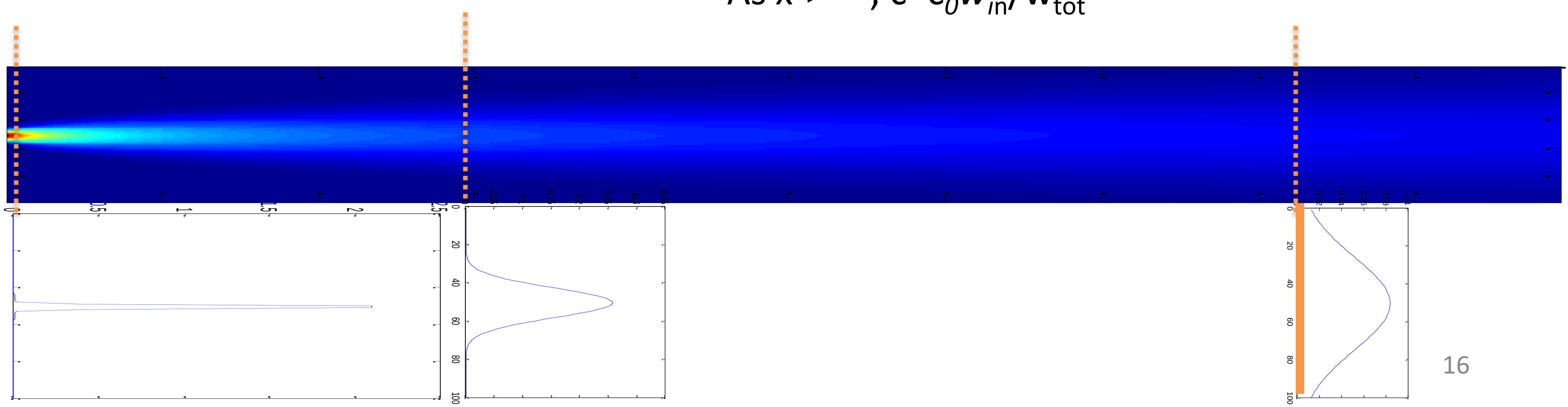
- Convective Diffusion $0 = \mathcal{D}\frac{\partial^2 c}{\partial^2 y} - v\frac{\partial c}{\partial x}$
  - Assumptions:
    - Very “spiky” initial concentration
    - Infinite room to diffuse into
  - Solution: $c = \frac{c_0 FRR/w_{tot}}{\sqrt{4\,\pi\, xD/v}} e^{-\frac{y^2}{4x\,D/v}}$



Micro Fluid Reactor Design

- Convective Diffusion $0 = \mathcal{D}\frac{\partial^2 c}{\partial^2 y} - v\frac{\partial c}{\partial x}$
  - Assumptions:
    - Very “spiky” initial concentration
    - ~~Infinite room to diffuse into~~
  - Solution: $c = c_0 w_{in}\left[\frac{1}{\sqrt{4\,\pi\,xD/v}}e^{-\frac{y^2}{4x\,D/v}} + erfc\left(\frac{w_{tot}/2}{\sqrt{4xD/v}}\right)\cdot\frac{1}{w_{tot}}\right]$

As x-> ∞, $c=c_0 w_{in}/w_{tot}$



Calculation Summary

$$\left\{\begin{aligned} \frac{\partial c}{\partial t} &= \mathcal{D}\nabla^2 c + \vec{\boldsymbol{v}}(x,y)\cdot\vec{\boldsymbol{\nabla}}c \\ \rho(\vec{\boldsymbol{v}}\cdot\boldsymbol{\nabla})\vec{\boldsymbol{v}} &= -\boldsymbol{\nabla}P + \mu\nabla^2\vec{\boldsymbol{v}} \\ \nabla\cdot\vec{\boldsymbol{v}} &= 0 \end{aligned}\right\}$$

Valid everywhere

Need simulation software

$$c = \frac{c_0 w_{tot}/FRR}{\sqrt{4\,\pi\,xD/v}}\,e^{-\frac{y^2}{4x\,D/v}}$$

Good for $w_{tot} < x < \dfrac{w_{tot}}{2\sqrt{2D/v}}$

(≈500 w_{tot} for micelles)

$$c = c_0 w_{in}\left[\frac{1}{\sqrt{4\,\pi\,xD/v}}\,e^{-\frac{y^2}{4x\,D/v}} + erfc\left(\frac{w_{tot}/2}{\sqrt{4xD/v}}\right)\cdot\frac{1}{w_{tot}}\right]$$

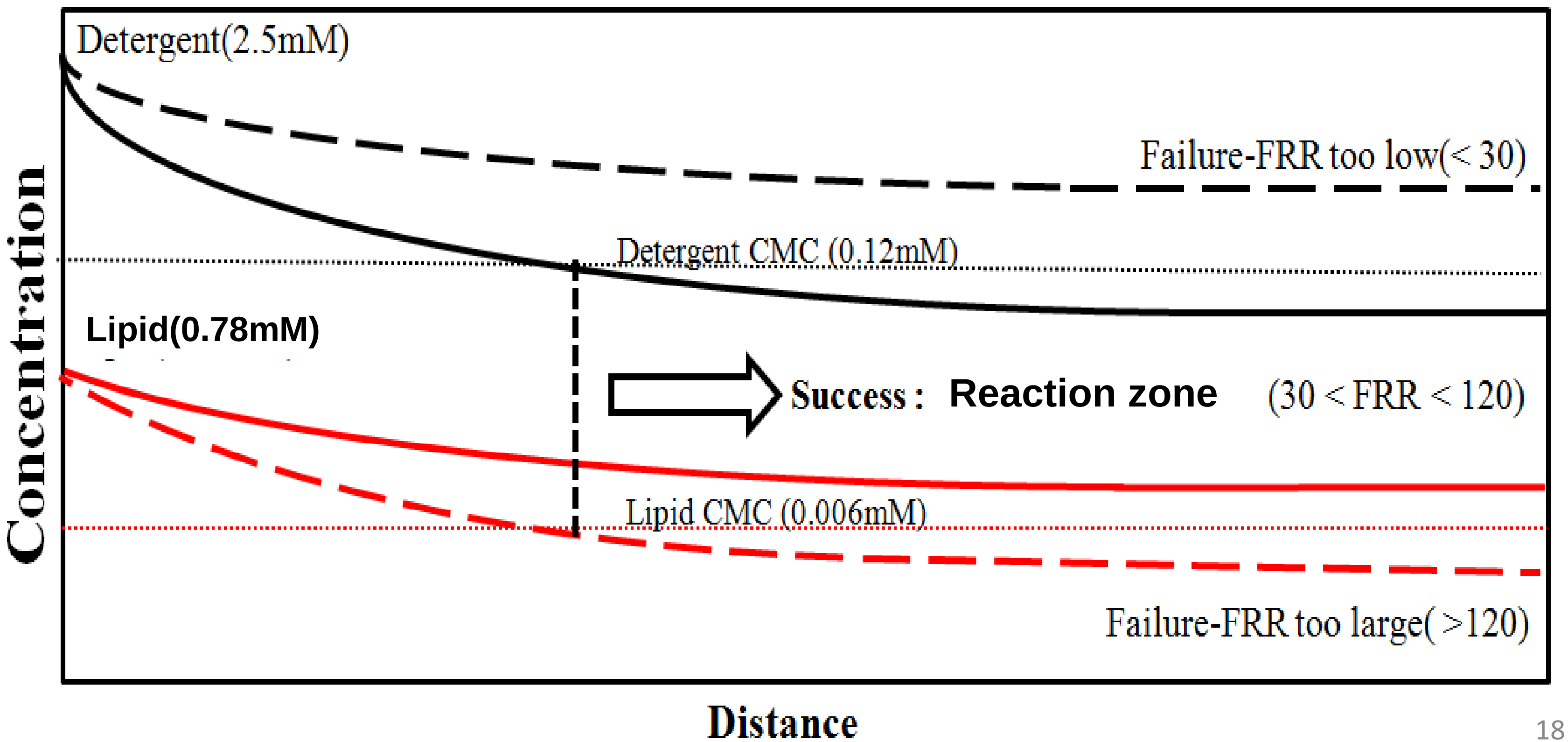
Good for $w_{tot} < x < \dfrac{(w_{tot}/0.01)^2}{4\pi D/v}$

$c = c_0 w_{in}/w_{tot} = c_0/FRR$

Good for $x > \dfrac{(w_{tot}/0.01)^2}{4\pi D/v}$

Micro Fluid Reactor Design

- What it means to be successful.
  - FRR tuned to force detergent monomers and lipid micelles at x->∞
  - Length long enough to allow reaction



Sample Calculation

	Lipid	Detergent
Initial Concentration	0.78 mM	2.5 mM
CMC	0.006 mM	0.17 mM
Diffusion coefficient	$3x10^{-8}$ cm^2/s	$7.5x10^{-9}$ cm^2/s
velocity	0.02 cm/s	
Total width	0.01 cm	

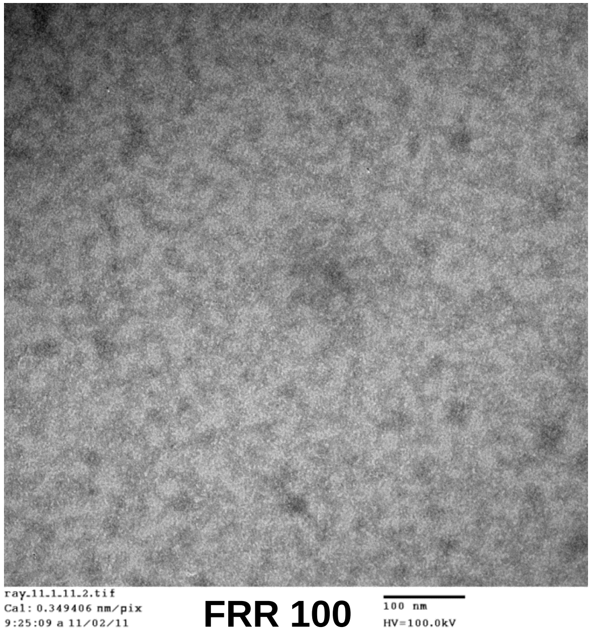
- What is the max FRR? What is the min FRR?
- How far down the line does the reaction zone start? (using $c = \frac{c_0 w_{tot}/FRR}{\sqrt{4\pi xD/v}} e^{-\frac{y^2}{4x D/v}}$ and a mid-point FRR)

Workshop

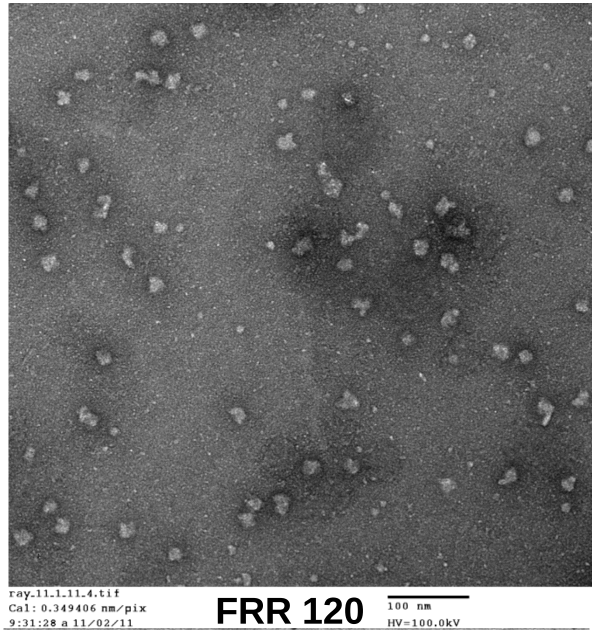
- Do the same calculations for a detergent whose CMC is 0.05 mM.
- How does increased velocity change things?
- What would happen if the detergent had a much larger diffusion coefficient?

Experimental Results

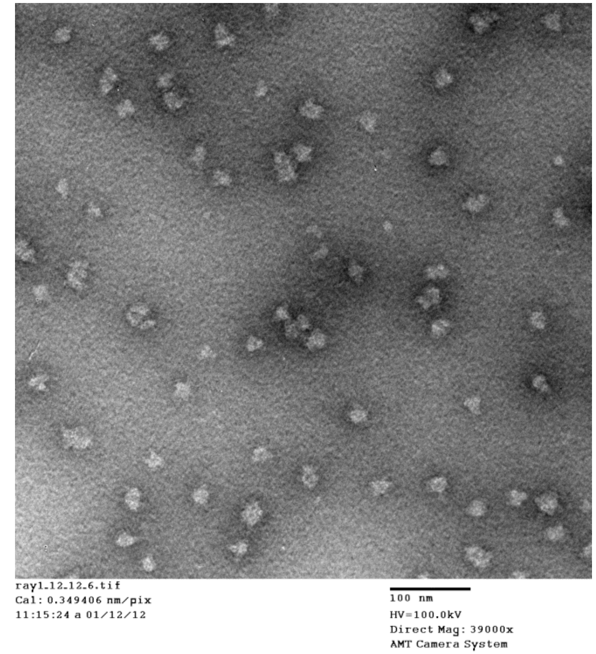
FRR 20



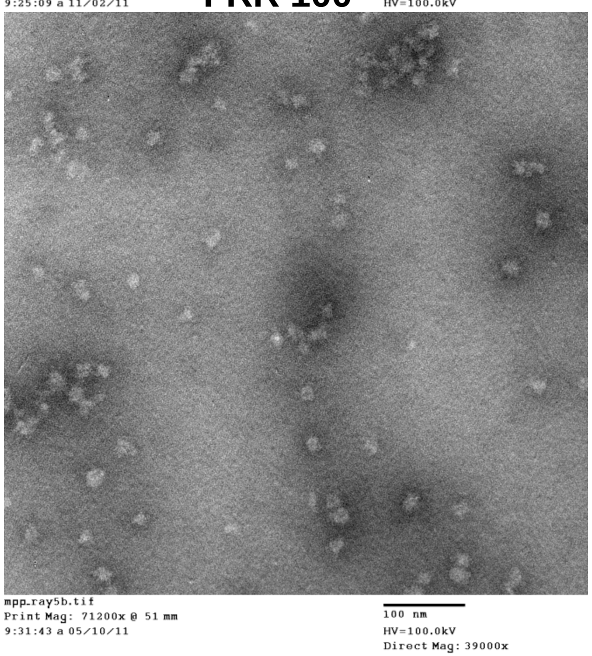
FRR 40



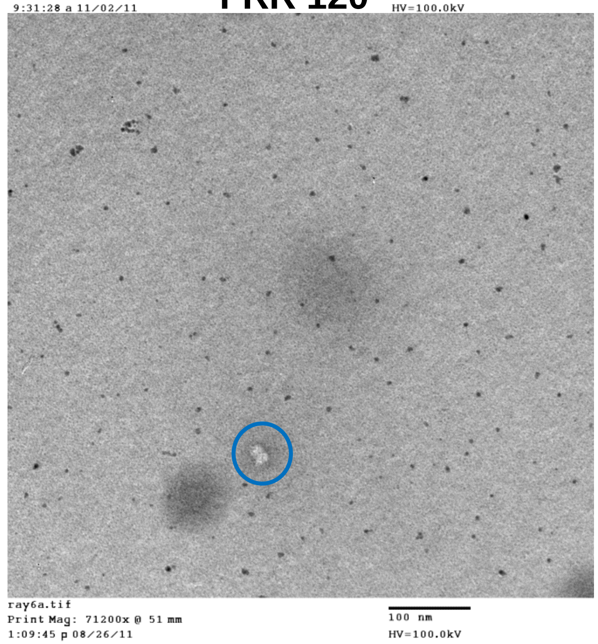
FRR 60



FRR 100



FRR 120

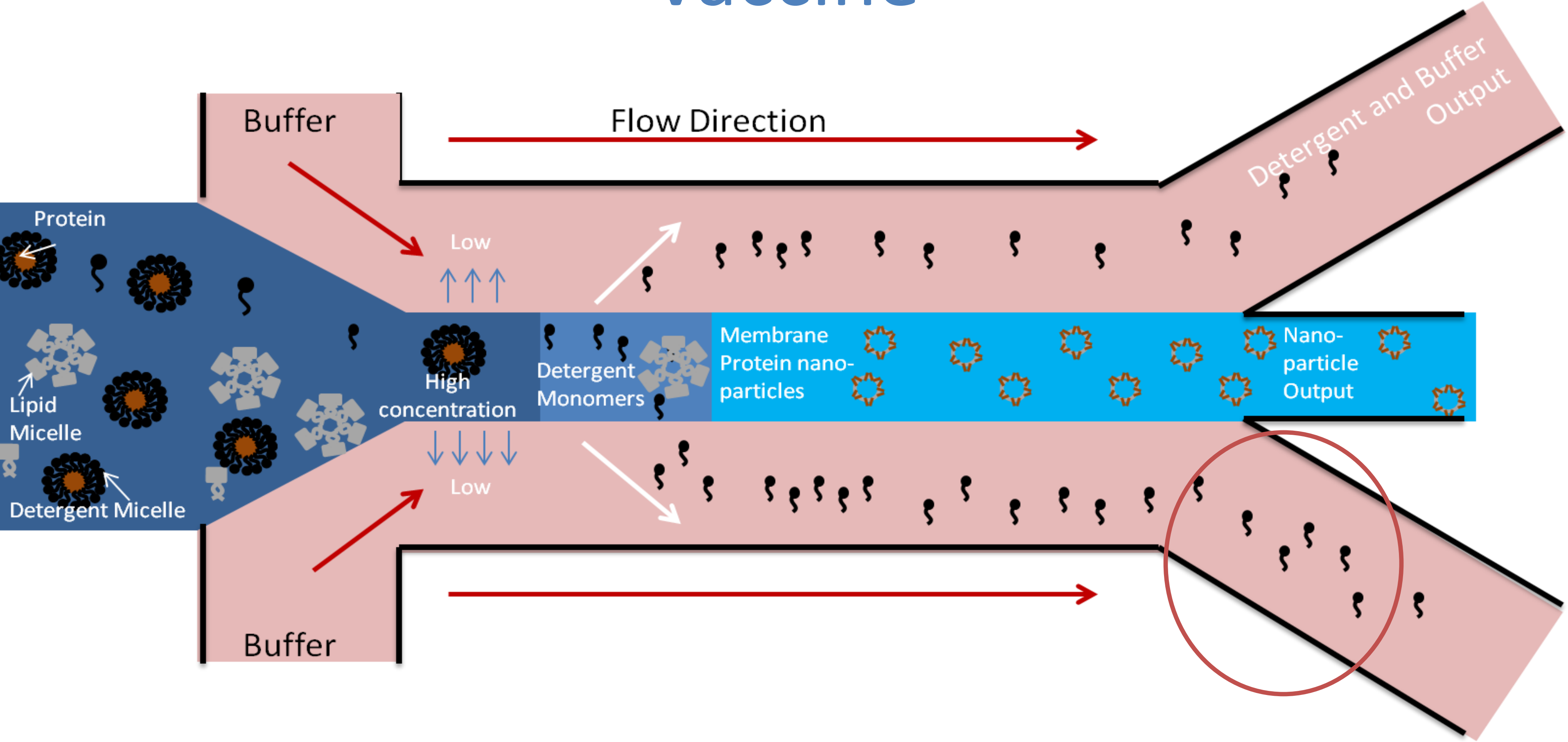


MPP only observed
FRR 30 < MPP < FRR 130

No MPP –
When FRR < 30,
Detergent FC-14 above CMC.

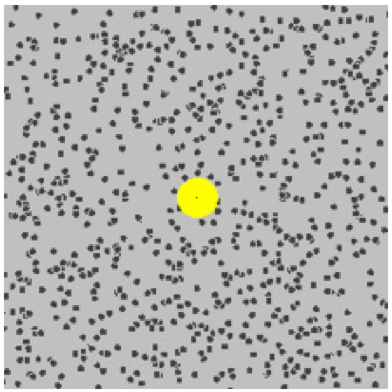
When FRR > 130,
Lipid PC-14 below CMC.

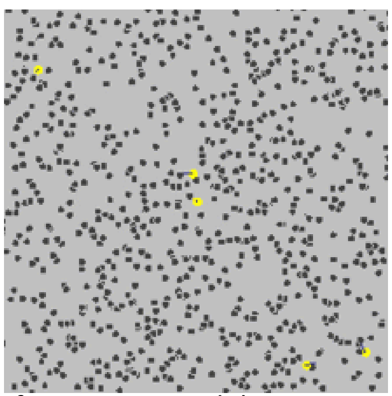
Bio/Micro Engineered Universal Flu Vaccine



Removing Monomers

- Small particles diffuse faster





Vertical distance traveled by random walk:

$$< y^2 > = \mathcal{D} \cdot t$$

Einstein-Stokes Relation

$$D = \frac{k_{\mathrm{B}} T}{6\pi \eta r}$$

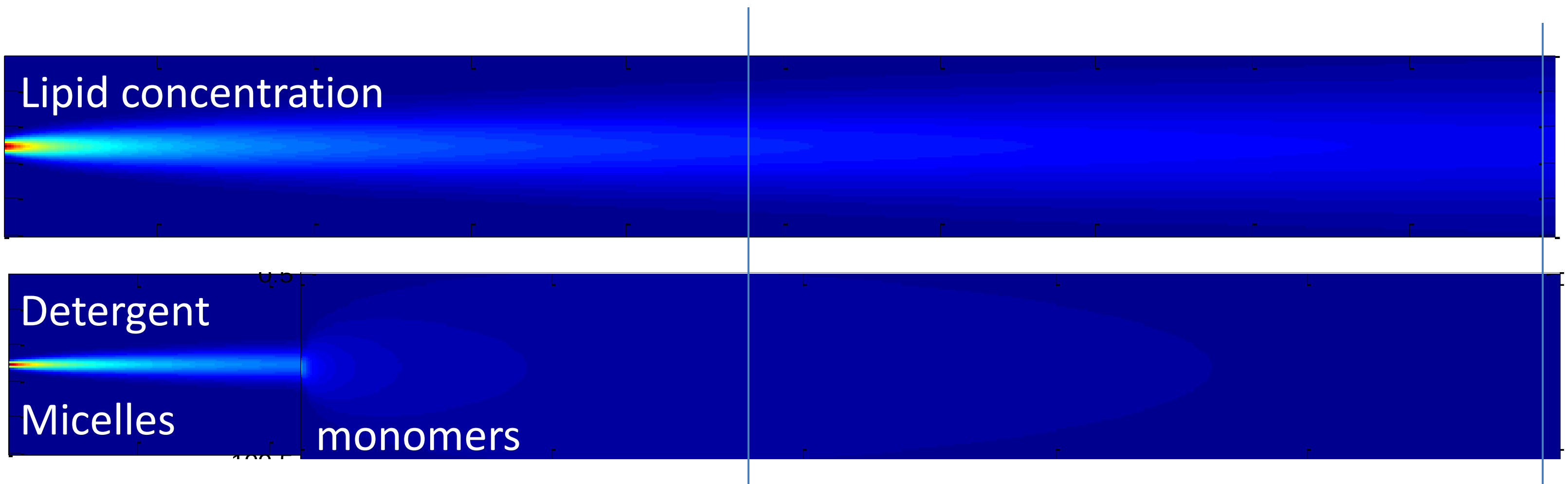
[Lookang](http://weelookang.blogspot.com/2010/06/ejs-open-source-brownian-motion-gas.html) Author of computer model: Francisco Esquembre, Fu-Kwun and lookang

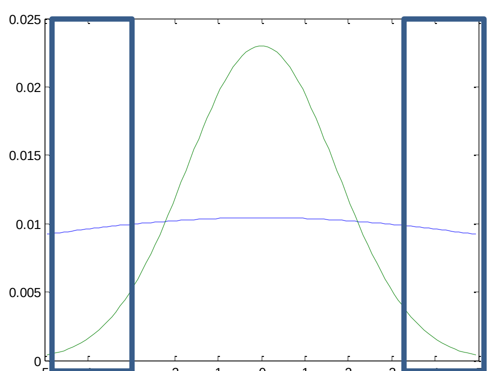
This is a simulation of Brownian motion of a big particle (dust particle) that collides with a large set of smaller particles (molecules of a gas) which move with different velocities in different random directions.

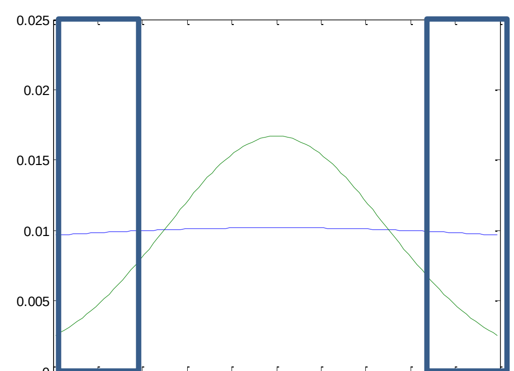
http://weelookang.blogspot.com/2010/06/ejs-open-source-brownian-motion-gas.html

Removing Monomers

- Detergent monomers diffuse to walls much faster



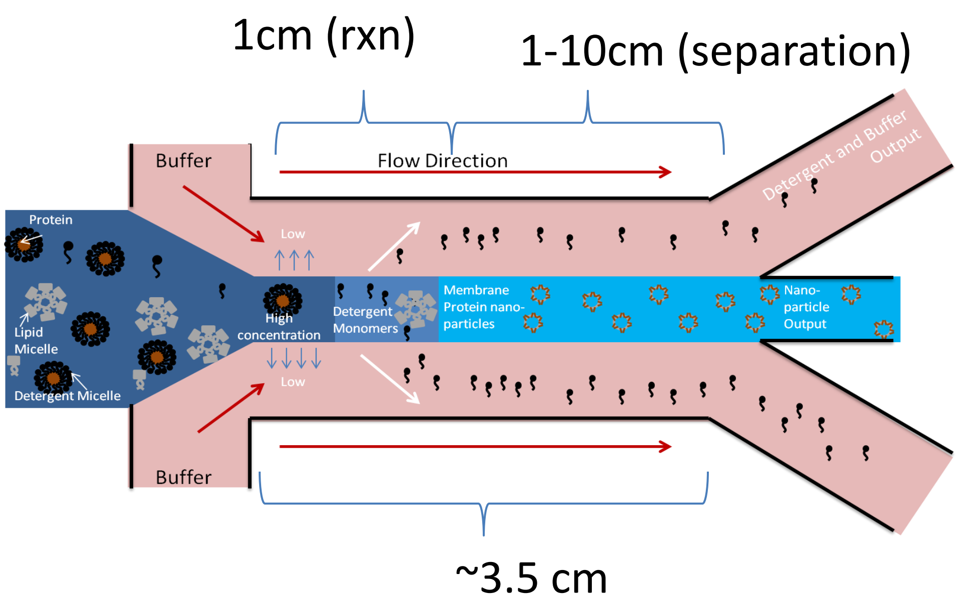




Removing Monomers

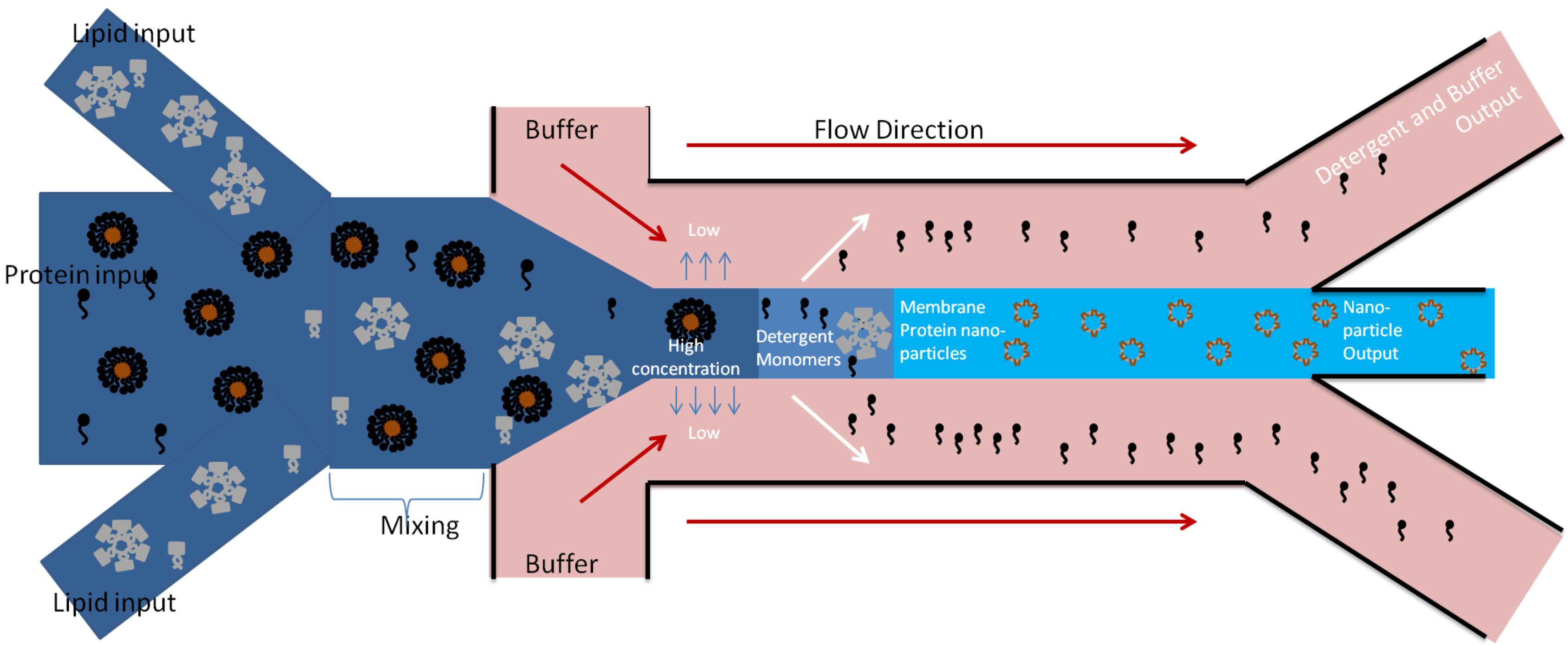
$t = y^2/D$
$x = vt$

$y^2 v/D_{mon} > x > y^2 v/D_{micelle}$
1 cm > *x* > 10 cm



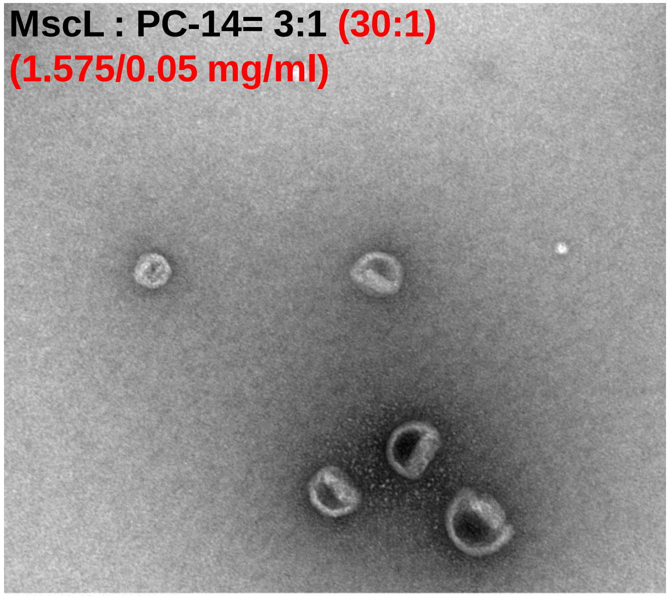
Protein-Lipid Ratios

Nanoparticle = *f* (***protein to lipid ratio,*** *ph, salt concentration, buffer solution****…)***



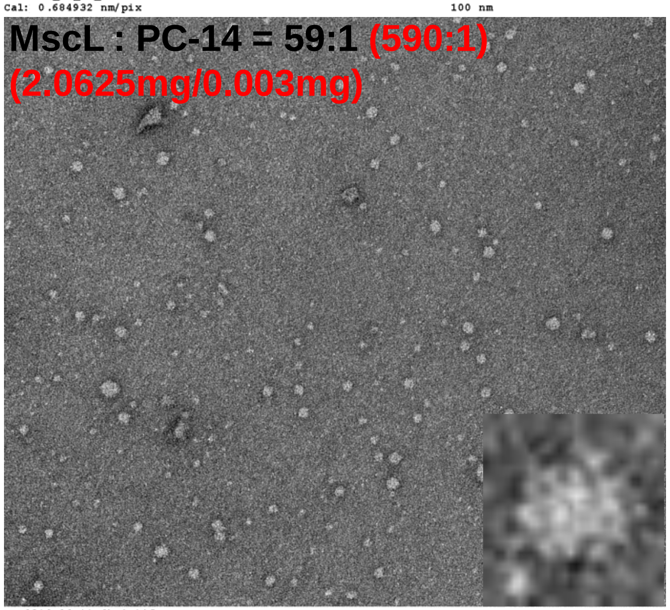
Experiment result -- We got MscL nanoparticles

MscL : PC-14= 3:1 (30:1) (1.575/0.05 mg/ml)



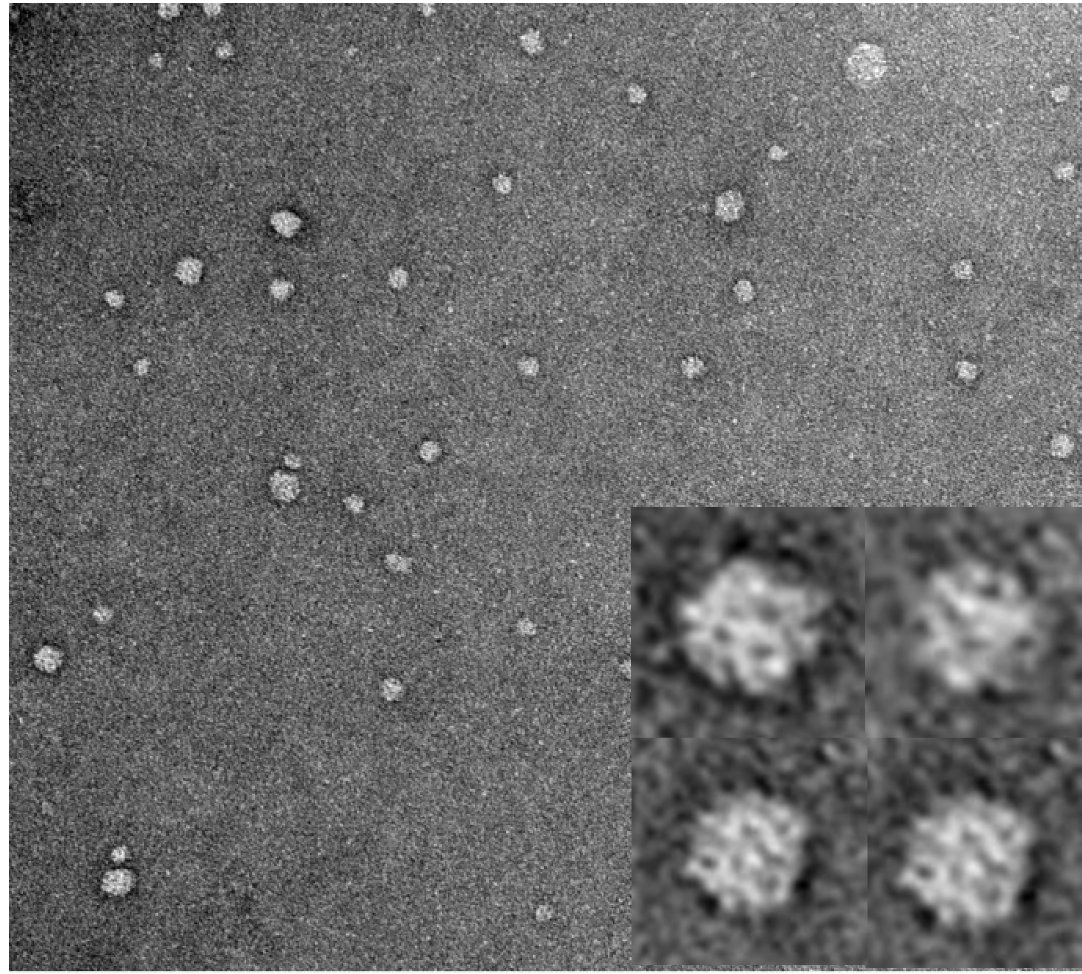
MPP2013_02_27_2b.tif
Cal: 0.684932 nm/pix
100 nm

MscL : PC-14 = 59:1 (590:1) (2.0625mg/0.003mg)



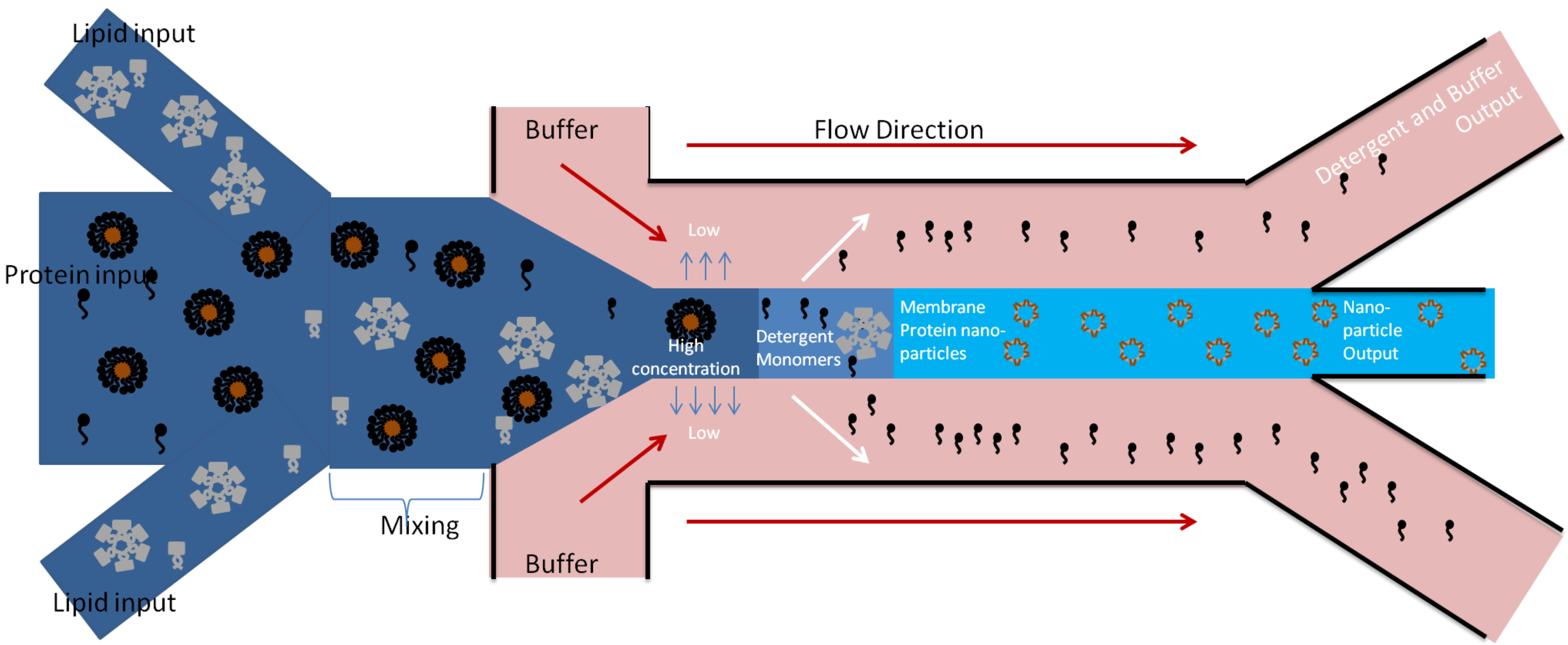
MPP2013_03_11_6b-1.tif
Cal: 0.684932 nm/pix
3:34:47 PM 3/12/2013
100 nm
HV=80.0kV
Direct Mag: 130000x
CM10-Dusty

MscL : PC-14 = 29:1 (290:1) (2.03/0.006 mg/ml)



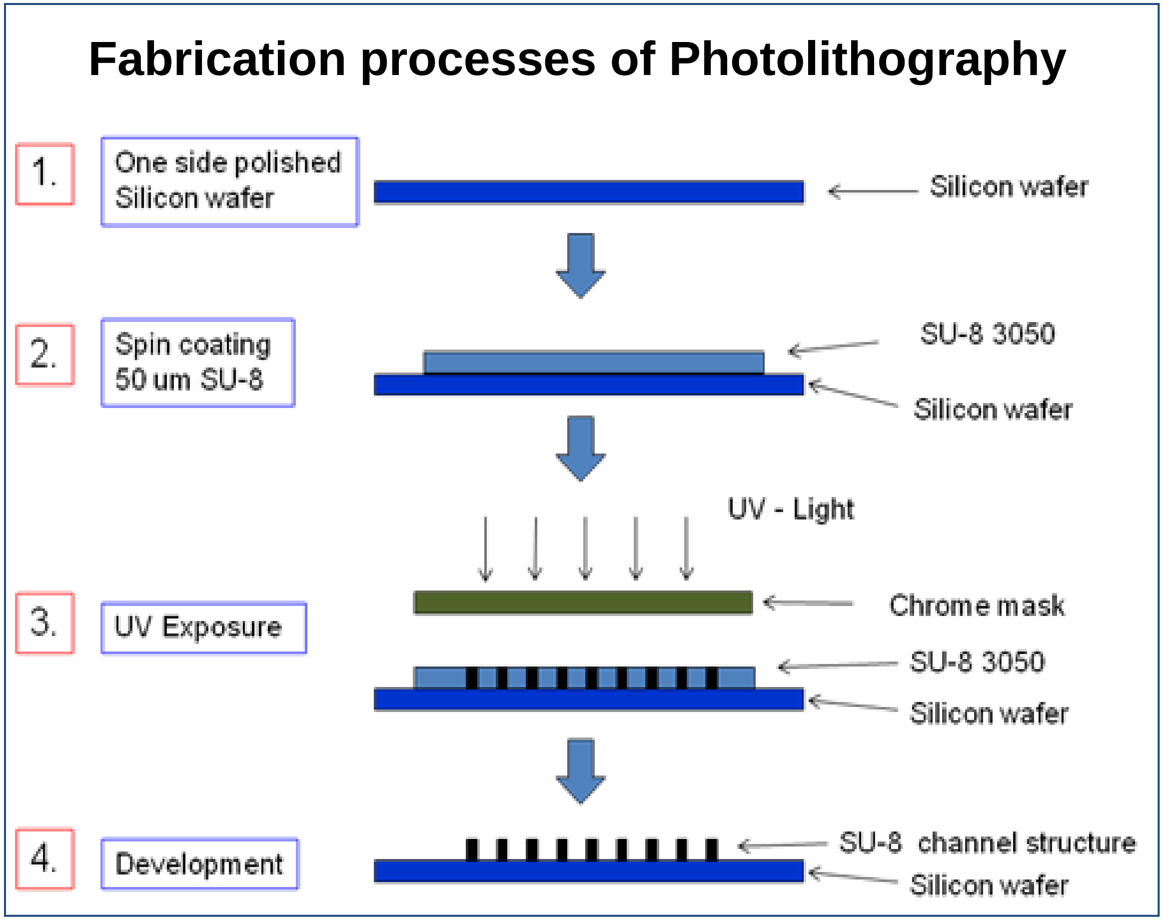
MPP2013_02_27_6b.tif
Cal: 0.684932 nm/pix
3:50:29 PM 3/1/2013
100 nm
HV=80.0kV
Direct Mag: 130000x
CM10-Dusty

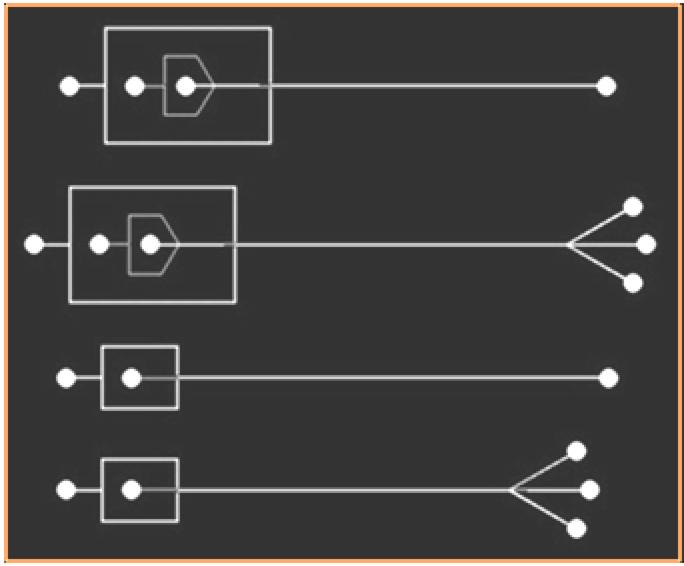
Device Microfabrication



Device Microfabrication

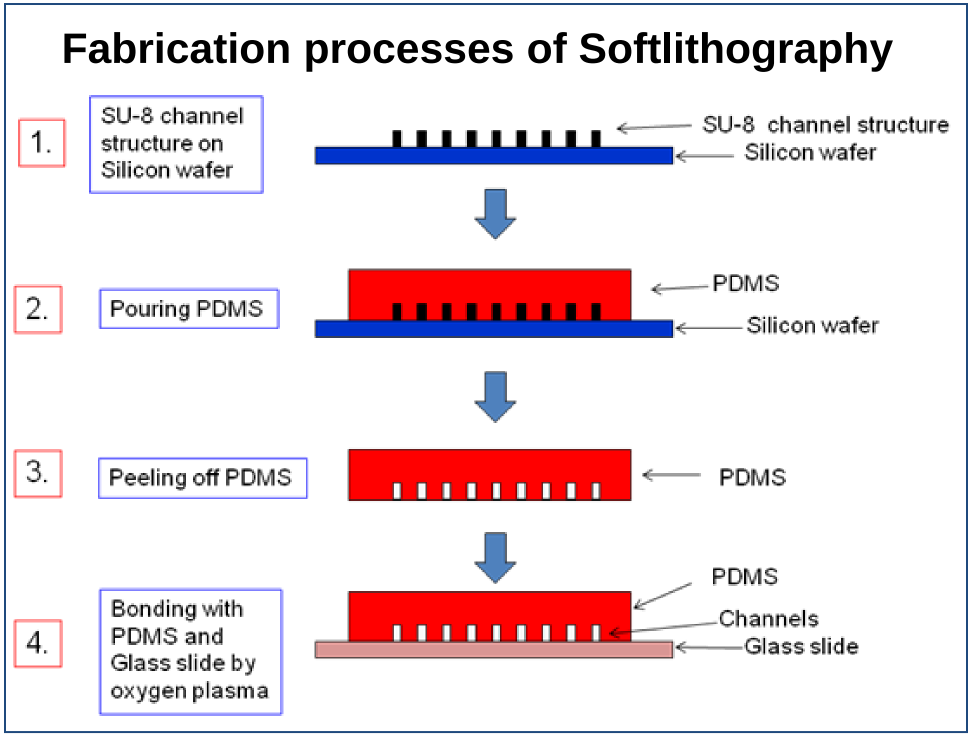
- Step 1: photolithography



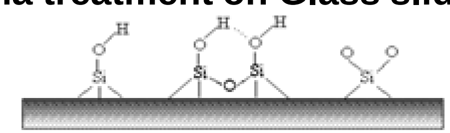


Device Microfabrication

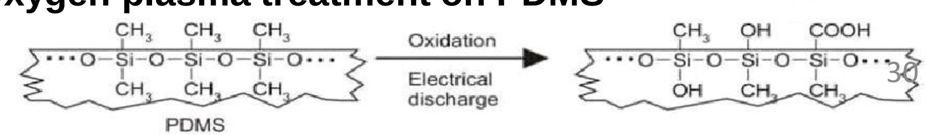
- Step 2: PDMS Soft Lithography



a. Oxygen plasma treatment on Glass slide



b. Oxygen plasma treatment on PDMS

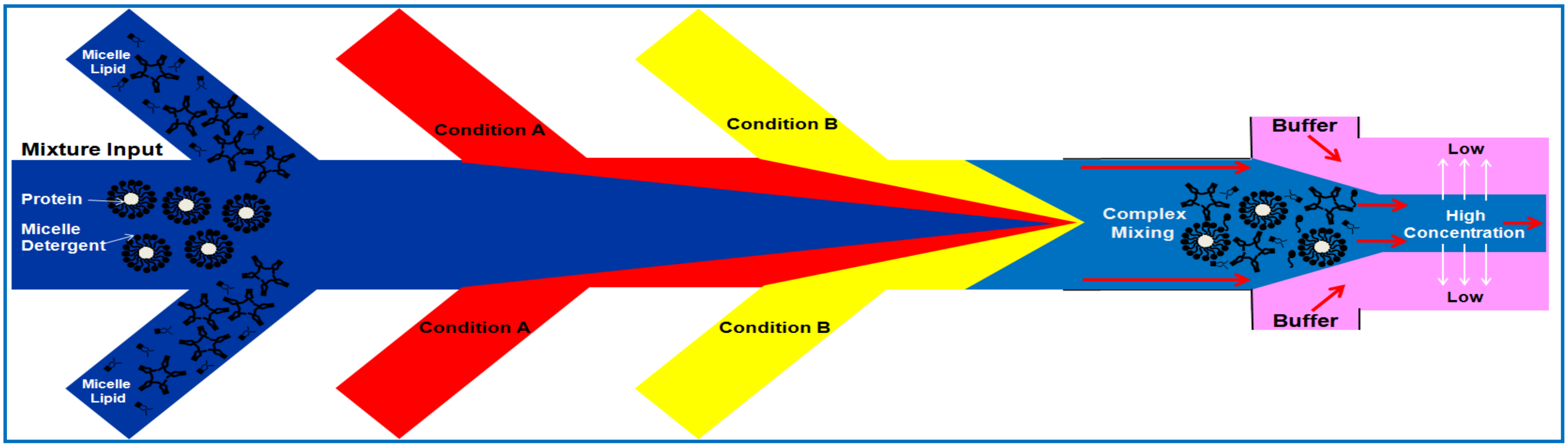


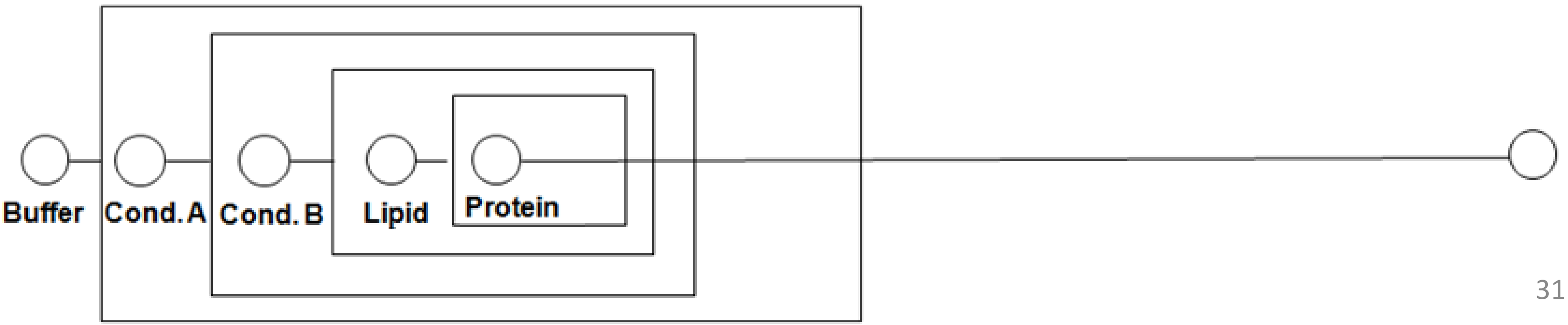
Future work

Improvement of microfluidic devices

Expend more inputs to test multi-condition.

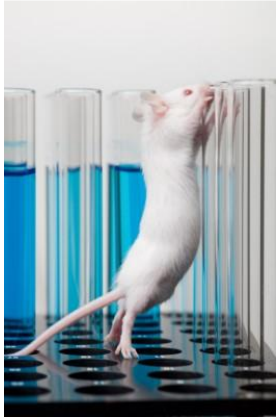
Nanoparticle = *f (protein to lipid ratio, ph, salt concentration, buffer solution…)*





After Micro-Engineering

- Test M2 Membrane-Protein Nano-particle as antigen in mice.



http://www.isumagazine.com/2011/09/to-conduct-animal-research-or-not/

- Use microfluidics to zero-in on conditions to generate antigen MPN's.
- Manufacture MPN's using dialysis in industrial-scale drums.

Conclusions

- Nanoparticles for a universal flu vaccine
- Diffusion-based microreactors for nanoparticles
- Physics of convection/diffusion for micro-reactor design
- Microfabrication based on reactor design