Predicting Subcutaneous Drug Absorption via Machine Learning Attempts and A Custom In Vitro Device

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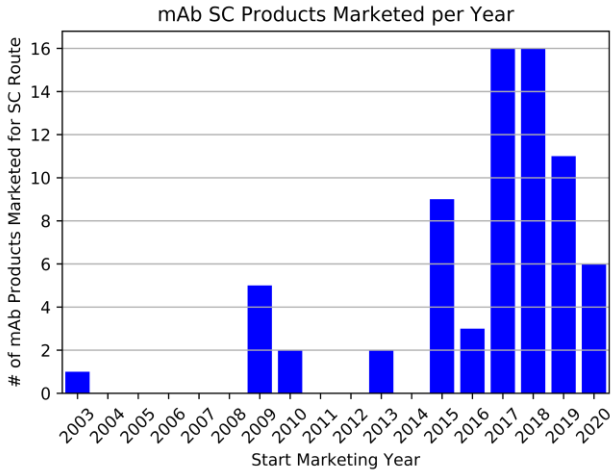
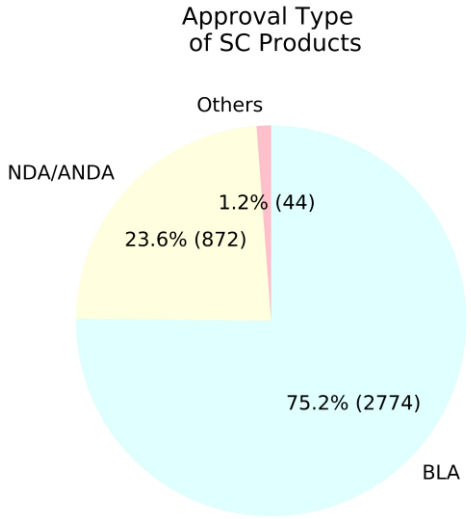
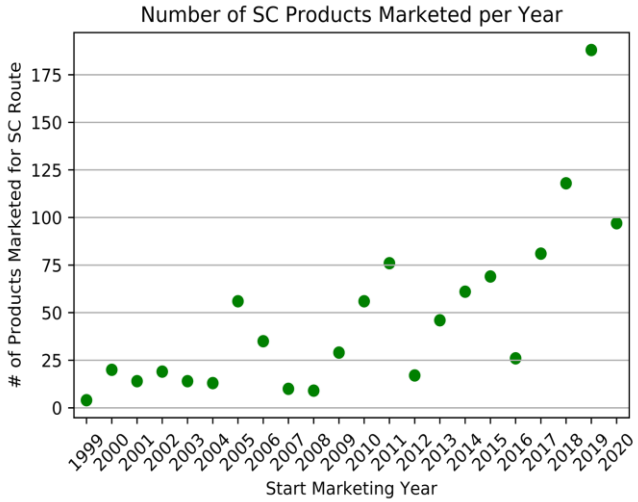
Current Status of SubQ Products

Analysis of FDA Database (National Drug Code Directory)







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Knowledge Gap

Unpredictable & Variable In-Vivo Properties

Humira® (Adalimumab) Bioavailability:

- Monkey (96%); Human (64%)

Herceptin® (Trastuzumab) Tmax:

- Mice (7 hours); Minipig (1 day); Human (4 days)

Lack of Models

- No reliable pre-clinical animal models

Methods to Study SubQ Drug Delivery

Open the Blackbox?

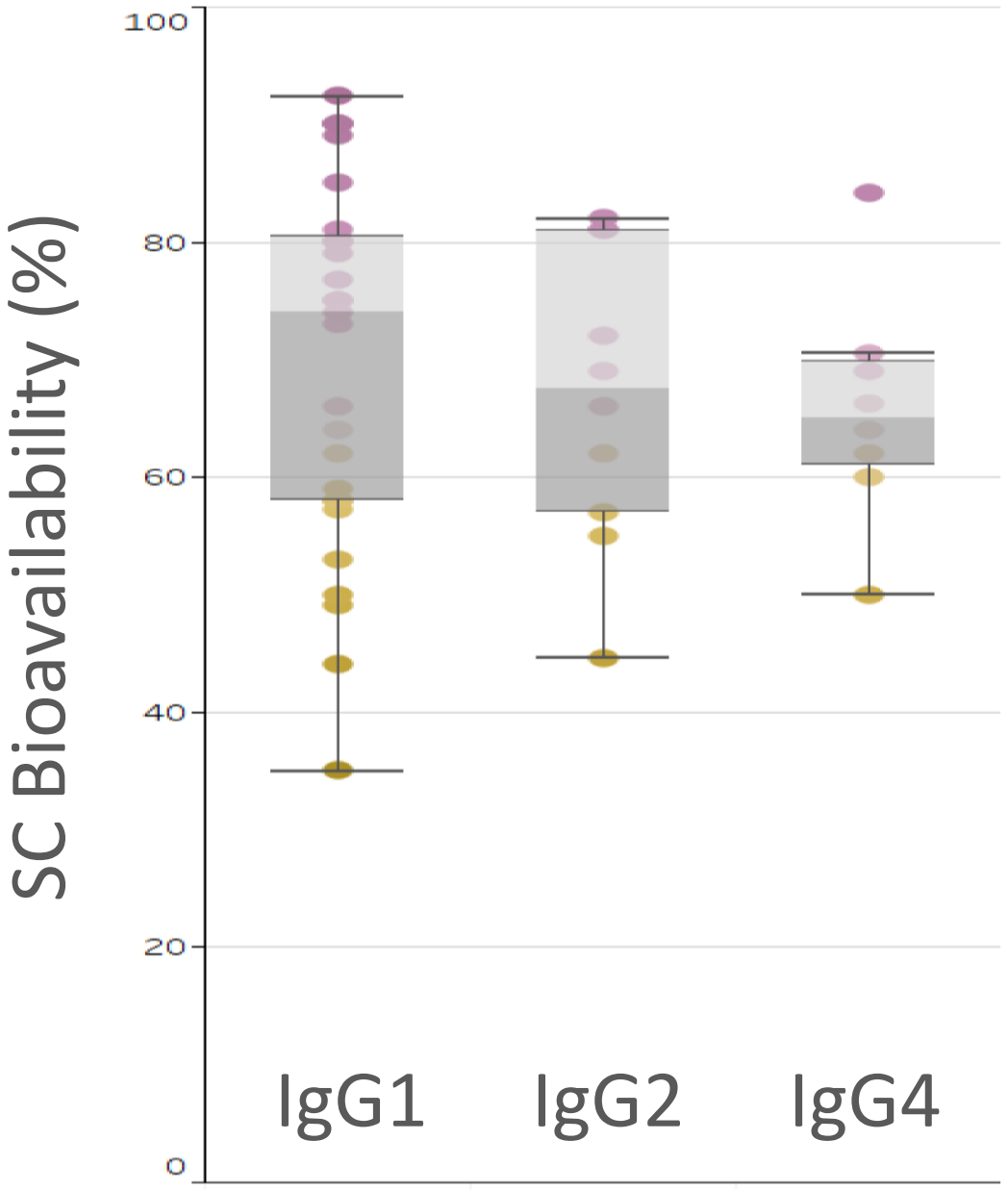
No

Not Study Mechanism

Machine Learning:
mAb SubQ
Bioavailability
Prediction

Machine Learning to Predict mAb Bioavailability

- 45 mAb molecules from public databases
- Bioavailability data from clinical studies



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Feature Selection

- A total of 47 features related to molecules and formulations
- Features mostly obtained from computational calculation instead of experiments

29 Features obtained based on Sequence

Adalimumab

>Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLAWYQQKPGKAPKLLIYAASTLQS
GVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>Heavy Chain

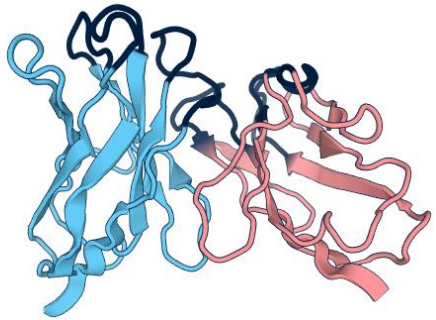
EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITW
NSGHIDYADSVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLD
YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

Examples of Features

- **# of Amino Acid**
- **Molecular Weight**
- **Theoretical pI**
- **Theoretical Charge @Ph7.4**
- **Hydropathicity (GRAVY)**
- **Aggregation Propensity**
- **CDR Length**
- **V_H/V_L Packing Angle**

…

16 Features obtained based on Ternary Structure



Ternary Structure of Fv Region (Computational Prediction)

Examples of Features

- **Energy per AA**
- **Charge per AA**
- **Dipole Moment**
- **Positive/Negative Patch Size**
- **Solvent Accessible Surface Area (SASA)**
- **SASA Polar**
- **SASA Apolar**

…

2 Features obtained based on Formulation

Published Literature; Filing Document; Insert, etc.

Examples of Features

- **Formulation Concentration**
- **Formulation pH**

Some important information (e.g., viscosity, glycosylation pattern, etc.) are missing due to data confidentiality

Dimensionality Reduction

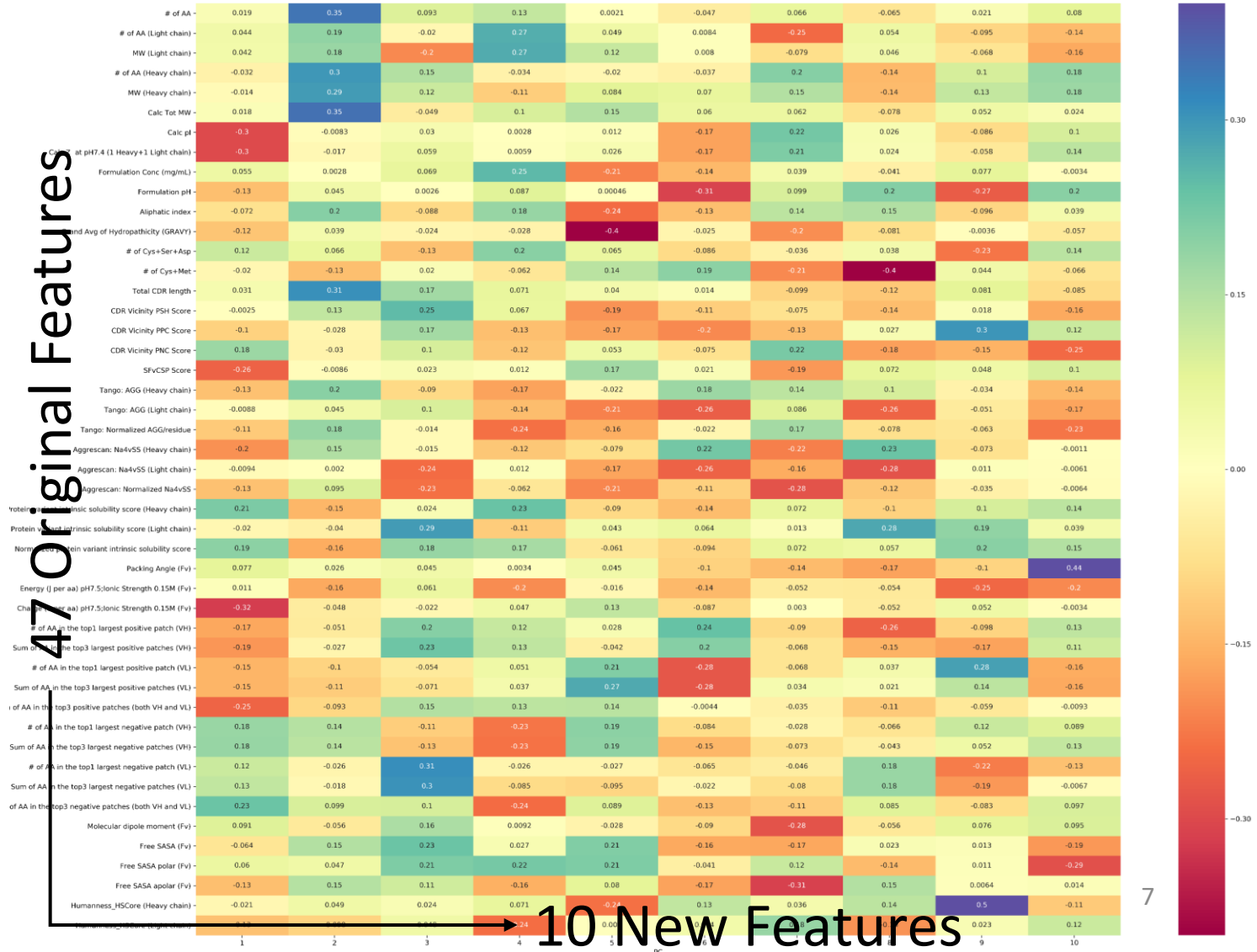
- Some features are correlated and redundant
- Reduce to 10 new features (PCs) from 47 features with 78% of the data variances retained

PC1 ⟺ **"Charge" Features**
- pI Value
- Charge@pH7.4
- Charge per AA
- Positive Patch Size

PC2 ⟺ **"Length" Features**
- # of AA
- MW
- CDR Length

PC3 ⟺ **"Hydrophobicity" Features**
- Aggregation Propensity
- Intrinsic Solubility
- Solvent Accessible Surface Area (SASA)

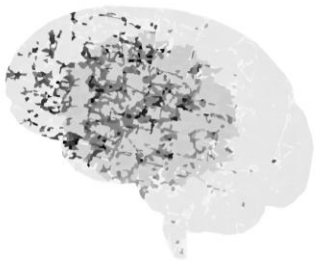
New Features — Old Features



mAb SC Bioavailability Prediction: ≥ or <70%

Machine Learning

Inputs:
mAb & Formulation Properties



Outputs:
Subcutaneous Bioavailability

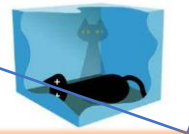
Machine learning algorithms	Training set accuracy (%)	Validation set accuracy (%)
RF	92	78
AdaBoost	89	78
DT	89	78
MLP	94	67
GaussianNB	75	67
kNN	72	67

Applicable to some early-stage research activities, e.g., mAb design & mutant screening

Lou H, Hageman M. Pharm Res 2021;38(3)

Methods to Study SubQ Drug Delivery

Open the Blackbox?
No / Yes



Not Study Mechanism

Study Mechanism

Machine Learning:
mAb SubQ
Bioavailability
Prediction

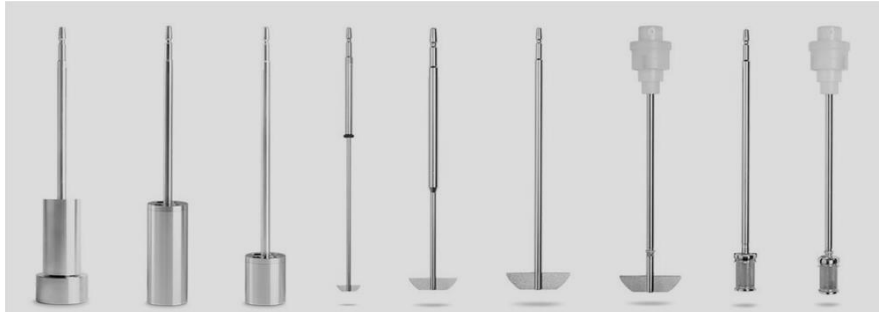
Multiple Mechanisms in an Integrated System

In-Vitro Models to Simulate SubQ Site

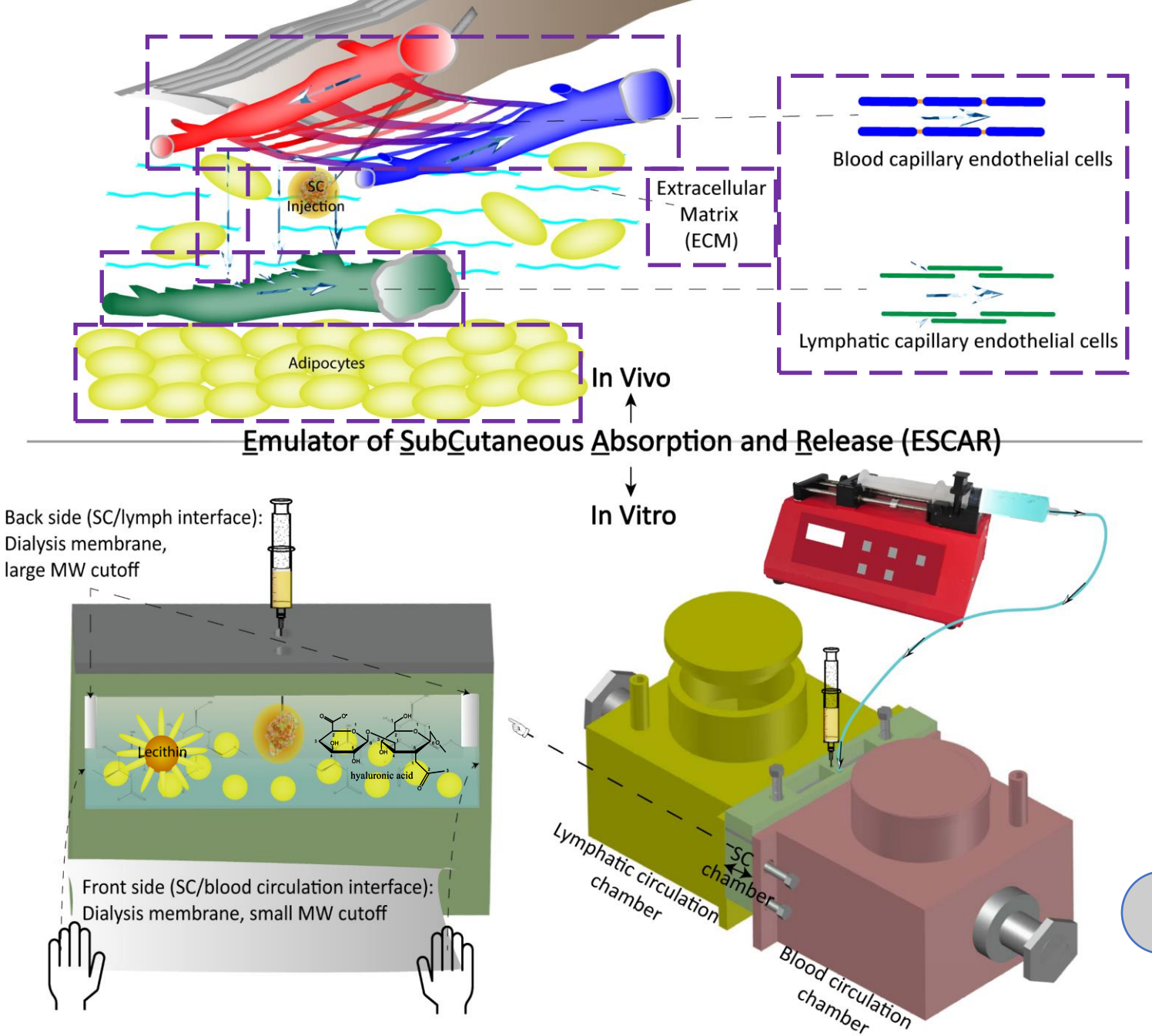
ESCAR
(Emulator of SubCutaneous Absorption and Release)

Oral vs. SubQ

USP Dissolution Apparatus:
Standard System for Oral Products



No Standard System
for SubQ Products



ESCAR Design

- Extracellular Matrix (hyaluronic acid)
- Fat Tissue
- Drug Uptake
- Tight Junctions
- Convection/Liquid Flow
- pH & Temperature & Ionic Strength

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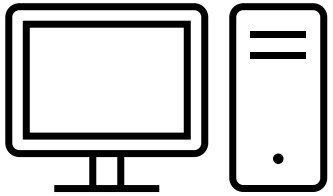
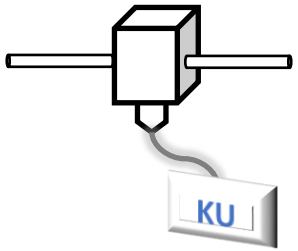
ESCAR Design & Fabrication



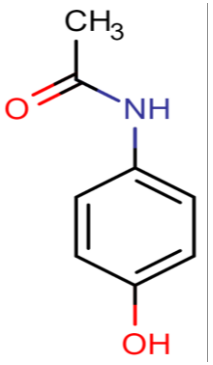
Computer-Aided Design



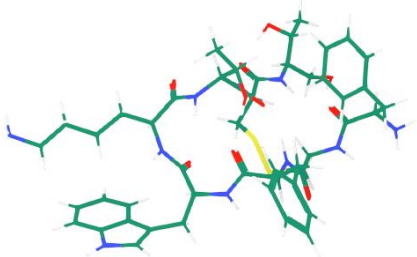
3D-Printing: Acrylonitrile Butadiene Styrene (ABS)

SC Chamber Design

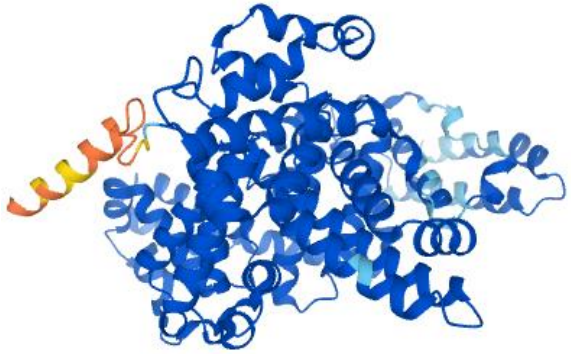
Application



Small Molecule



Peptide



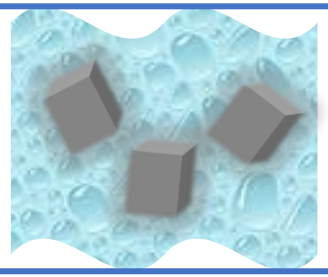
Protein

Formulation/DDS

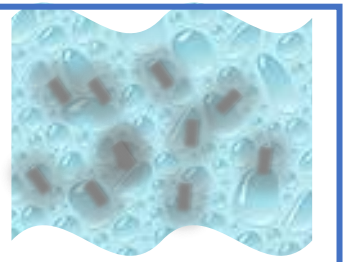
- Solution
- Suspension
- Gel
- Nanoparticle/Microparticle
- Depot System

In-Vitro-In-Vivo-Correlation (IVIVC): Griseofulvin Suspension

Formulation 1:
Micro-Suspension (UnMilled)

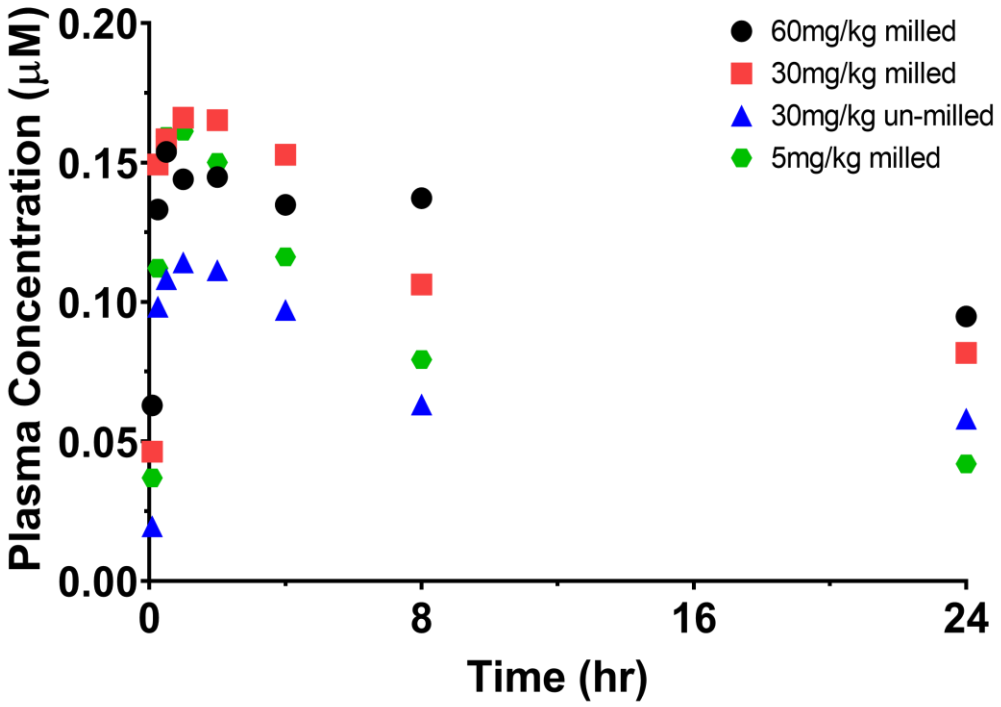


Formulation 2:
Nano-Suspension (Milled)



In Vivo

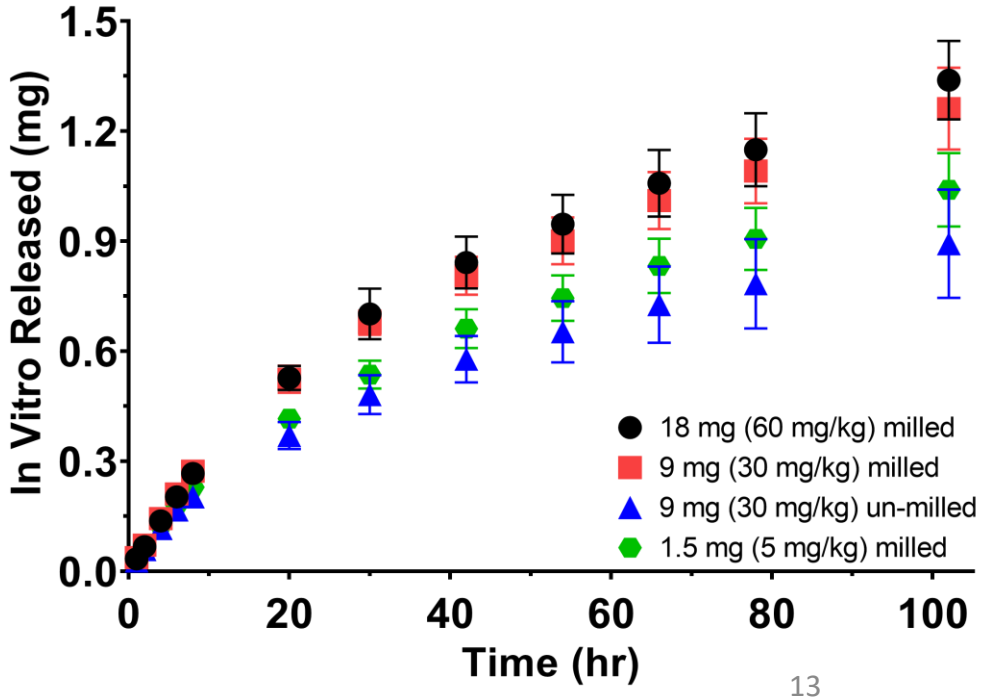
- Study Rat PK



In-Vivo can be predicted by In-Vitro?

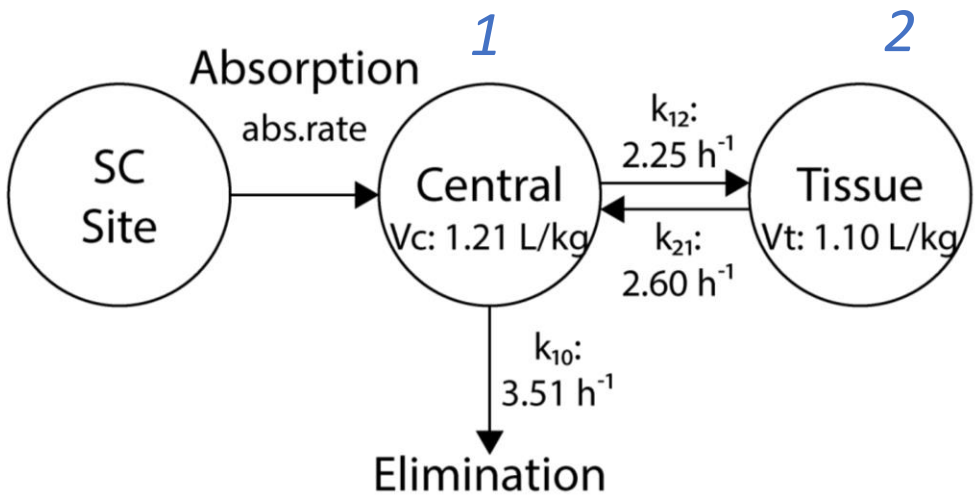
ESCAR

- Develop Drug Release Method (geometry, medium, condition, etc.)



Replotted from Chiang P-C, et al. J Pharm Sci. 2019;108(1).

Two-Compartment Model for Griseofulvin PK



$$\frac{dAmt1}{dt} = Abs.Rate - k_{10}Amt1 - k_{12}Amt1 + k_{21}Amt2$$

$$\frac{dAmt2}{dt} = k_{12}Amt1 - k_{21}Amt2$$

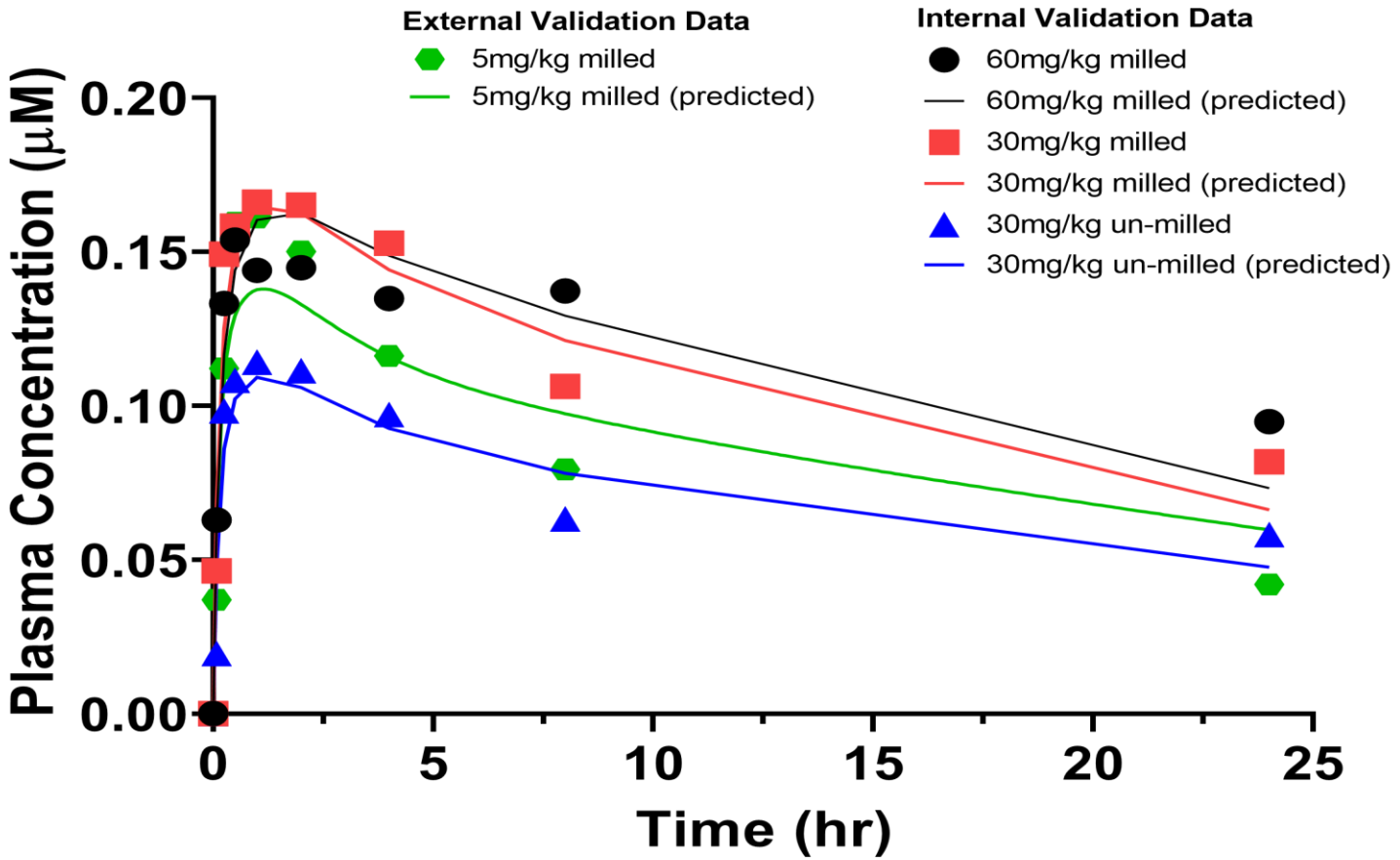
$$Conc1 = \frac{Amt1}{V_c}$$

Find a Correlation between Absorption Rate & Release Rate (In Vivo) (In Vitro)

$$Abs.Rate = \frac{Dose \times F(t + \Delta t|_{in\ vivo}) - Dose \times F(t|_{in\ vivo})}{\Delta t|_{in\ vivo}}$$

$$= \frac{Dose \times F(b_0 + b_1 \times (t + \Delta t) + b_2 \times (t + \Delta t)^2|_{in\ vitro}) - Dose \times F(b_0 + b_1 \times t + b_2 \times t^2|_{in\ vitro})}{b_0 + b_1 \times (t + \Delta t) + b_2 \times (t + \Delta t)^2|_{in\ vitro} - b_0 + b_1 \times t + b_2 \times t^2|_{in\ vitro}}$$

Lou H, Hageman MJ. Mol Pharm 2022.19(11)



Internal and External Validation for the IVIVC Model

Dose/Formulation	Data Type	Cmax (µM)			AUC0-24 (µM×h)		
		Obs.	Pred.	%PctErr	Obs.	Pred.	%PctErr
18 mg (60 mg/kg) milled	Internal	0.154	0.163	-5.7	2.95	2.77	6.1
9 mg (30 mg/kg) milled	Internal	0.166	0.165	0.9	2.64	2.63	0.5
9 mg (30 mg/kg) unmilled	Internal	0.114	0.109	4.4	1.71	1.74	-2.2
Average absolute %PE for internal validation				3.6			2.9
1.5 mg (5 mg/kg) milled	External	0.161	0.137	14.9	1.91	2.13	-11.5

SUMMARY of ESCAR & IVIVC (small molecule)

Model 1: ESCAR

- Reasonable emulation of in vivo SC environment

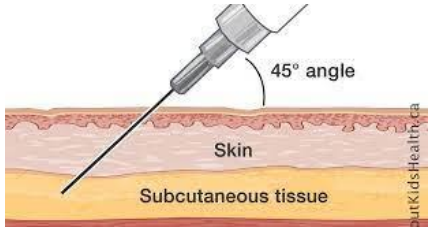
Model 2: In-Vitro-In-Vivo-Correlation (IVIVC)

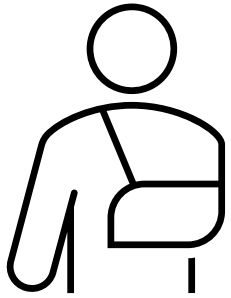
- Predict the PK profile of micro- and nano-suspensions for poorly water-soluble drug
- Reduce experimental efforts related to in vitro/in vivo studies

Critical Parameters of ESCAR (Acetaminophen 10 mg/mL Solution)

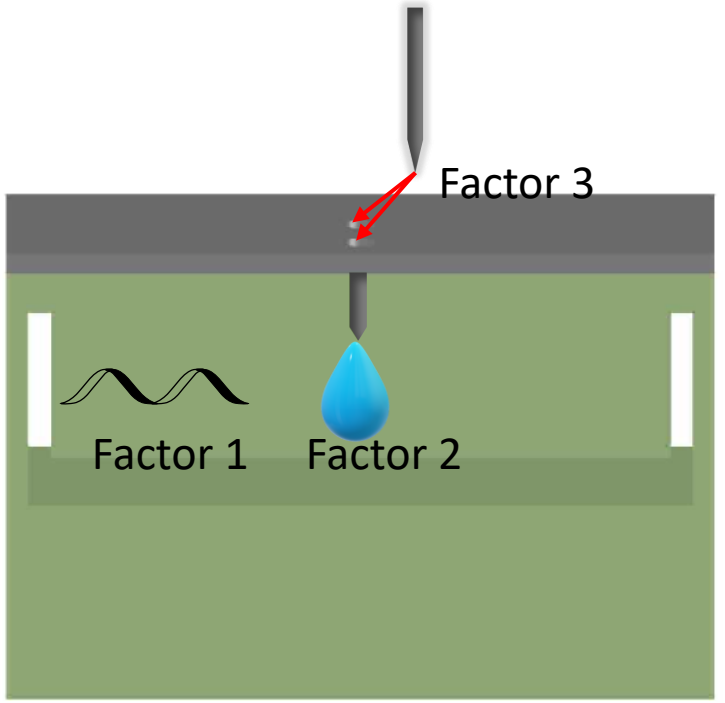
Factors may affect PK?

1. Hyaluronic Acid (HA)
   Level: Elder vs. Child
2. Dose: 5 mg vs. 10 mg
3. Injection Position



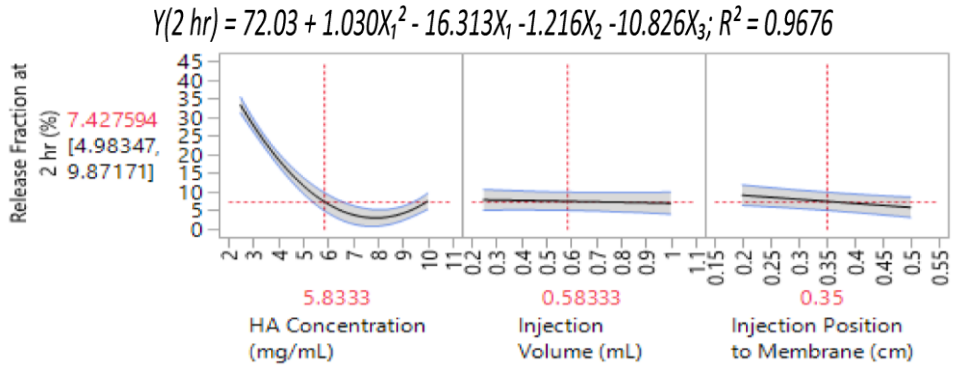


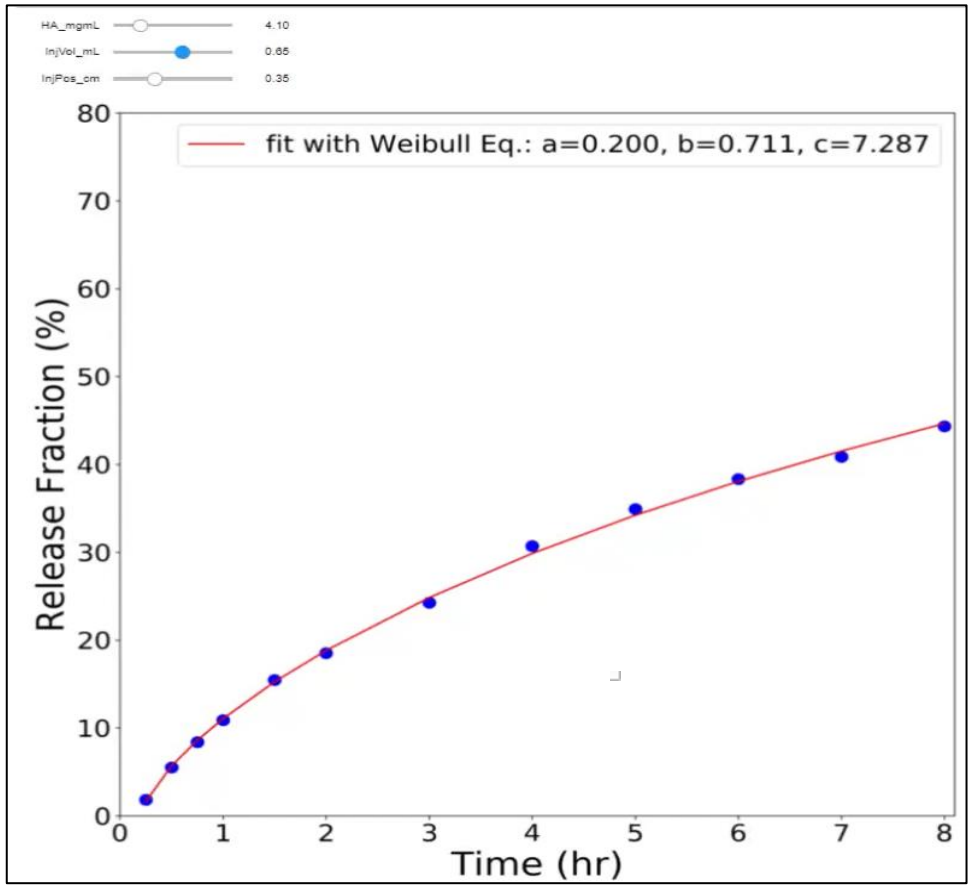
Hmm…
ESCAR?



Design-of-Experiment (DoE) Factor Screening 3×3×2=18 runs in triplicate	
Factor 1	HA Concentration (3 levels)
Factor 2	Dose/Injection Volume (3 levels)
Factor 3	Injection Position (2 levels)

Statistical Model vs. Machine Learning Model





Regression Score: R^2		
Predictive Models	**Y(2 hr) CrossValidation?**	
	No	**Yes: 4 Fold**
Polynomial Equation	0.9676	NA
Support Vector Machine (SVM)	0.9845	Dataset Training: 0.9944 Validation: 0.9283
Random Forest (RF)	0.9896	Dataset Training: 0.9882 Validation: 0.9146
Gradient Boosting	0.9960	Dataset Training: 0.9970 Validation: 0.9290
Multilayer Perceptron (MLP)	0.9976	Dataset Training: 0.9973 Validation: 0.9370

SUMMARY of DoE Study (small molecule)

Critical Parameters for Drug Release

- HA Concentration (Critical); Dose & Injection Position (Non-Critical)

Data-Driven Model

- Machine learning & statistical models can predict drug release
- Improve prediction of DoE data with convoluted pattern

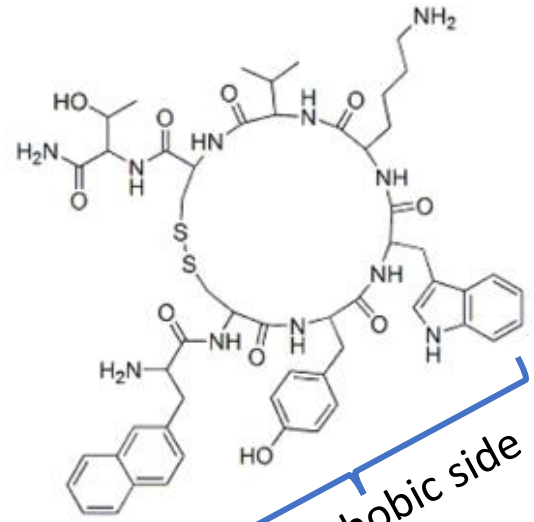
ESCAR Applications to Peptides

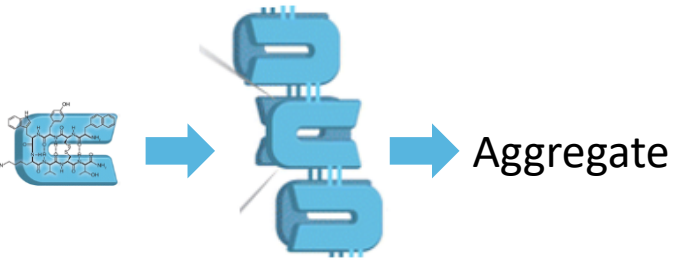
- High Concentration Peptide Formulation

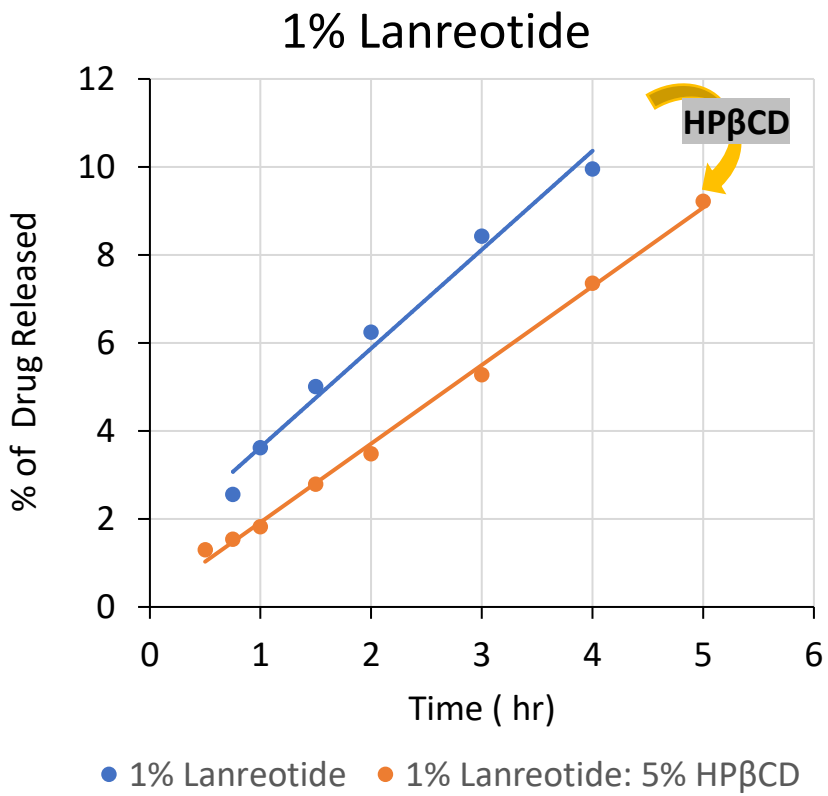
Facts we know:

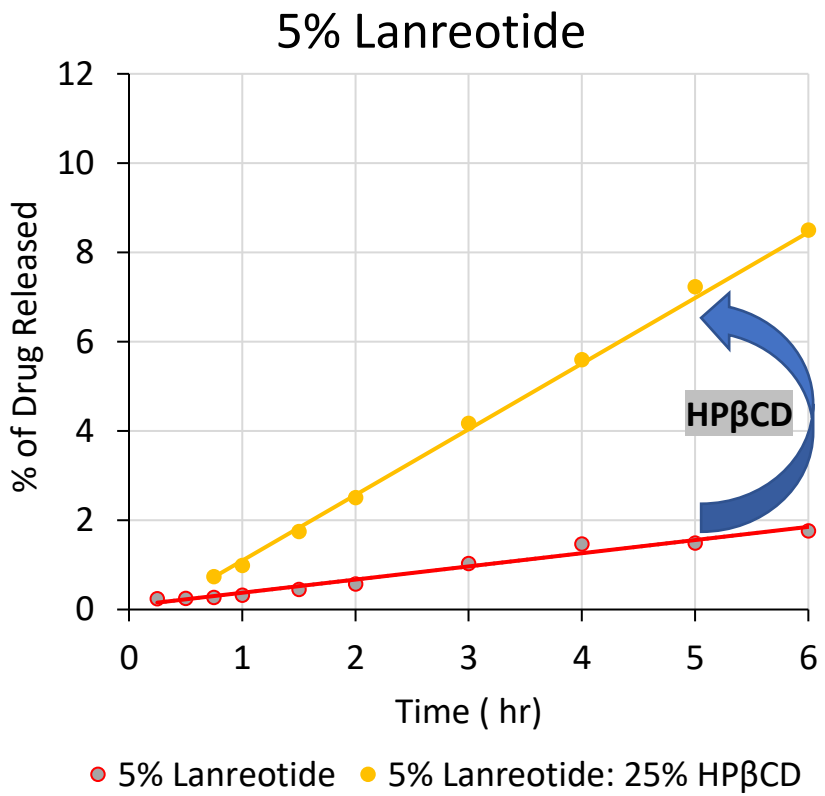
*Lanreotide aggregates above **3% w/v*** in water

Negar Jafari
PhD Candidate
KU Pharm Chem Dept

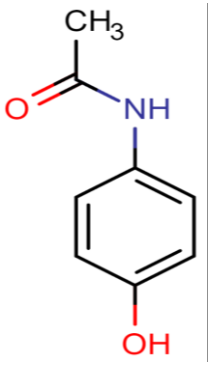






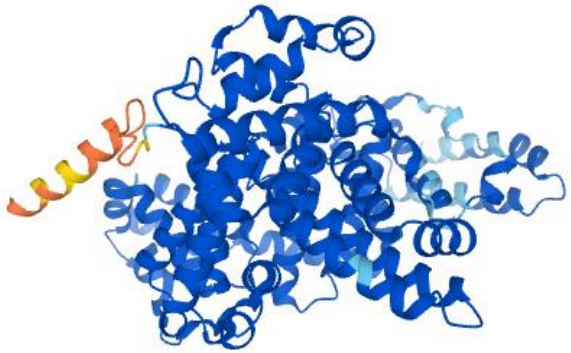


ESCAR Application to Proteins



Small Molecule & Peptide

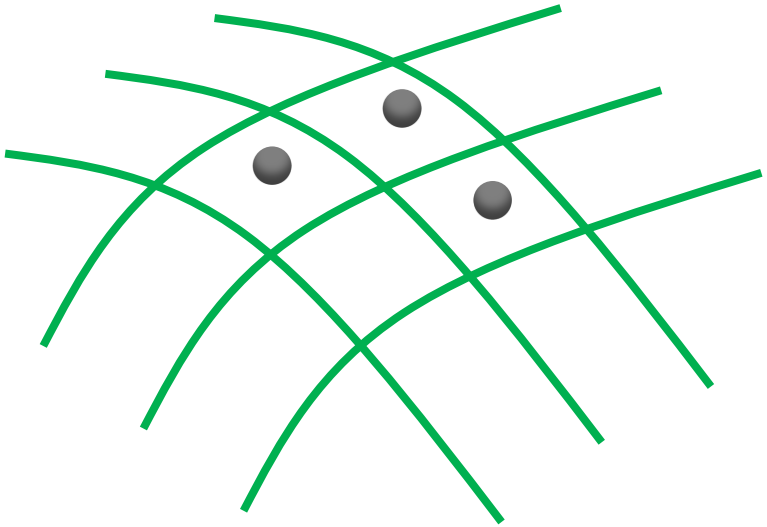
- ✓ Acetaminophen
- ✓ Griseofulvin
- ✓ Lanreotide



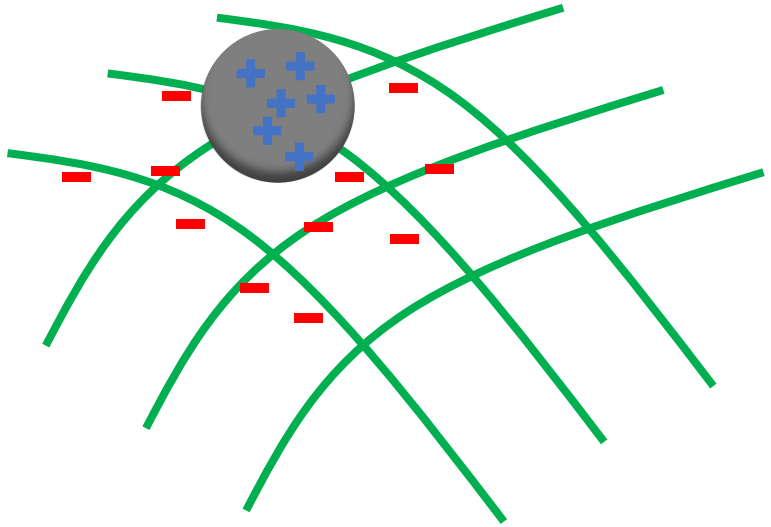
Protein

- ✓ Bovine Serum Albumin (BSA)

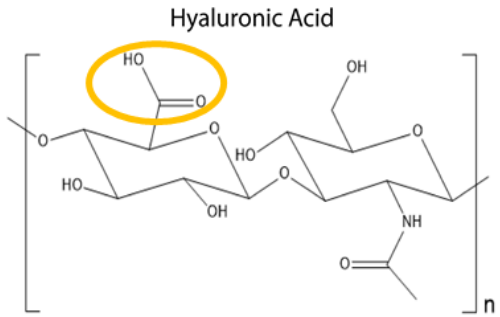
Molecule Diffusion in Hyaluronic Acid (HA) Solution



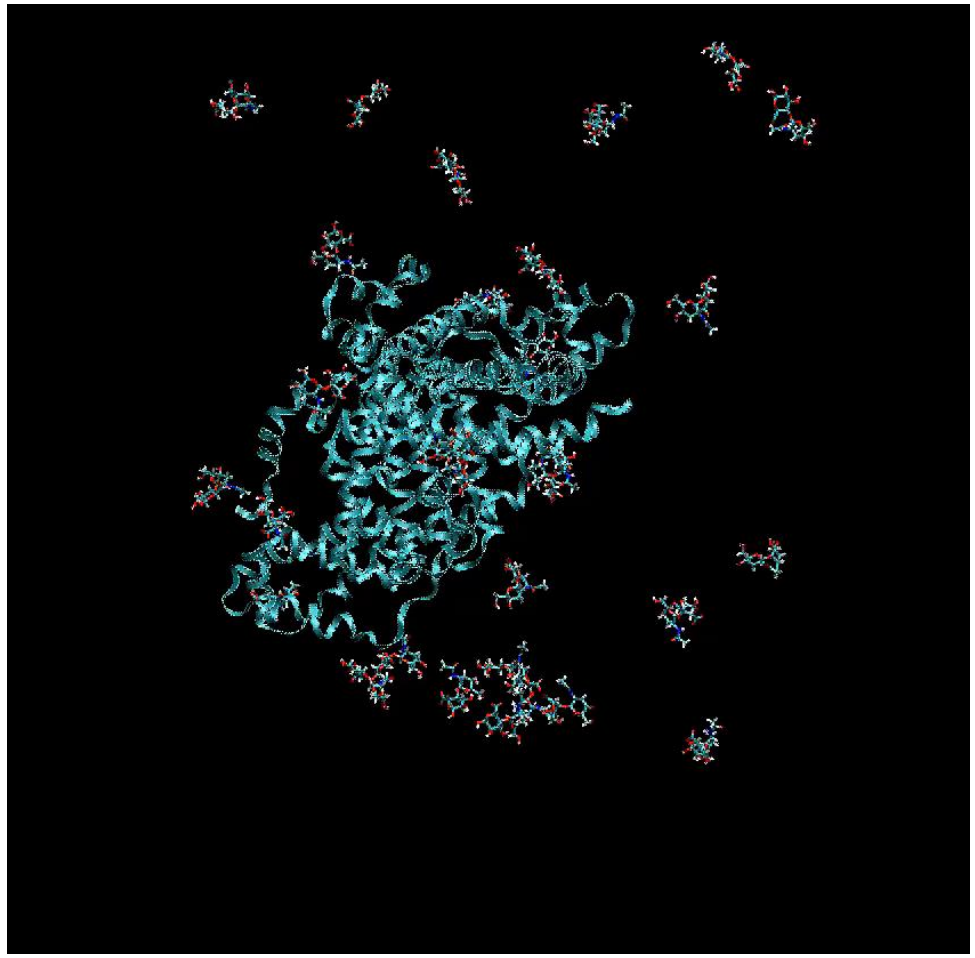
Small Molecule



Protein



BSA – HA Electrostatic Interaction



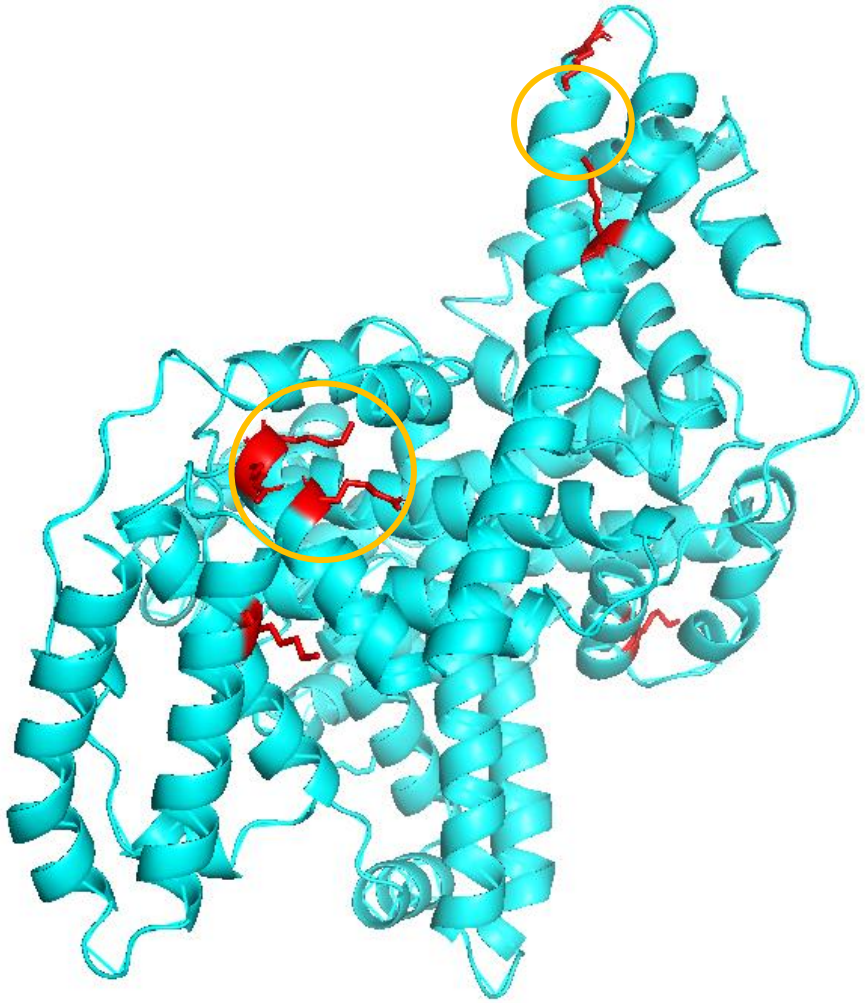
Molecular Dynamic Simulation

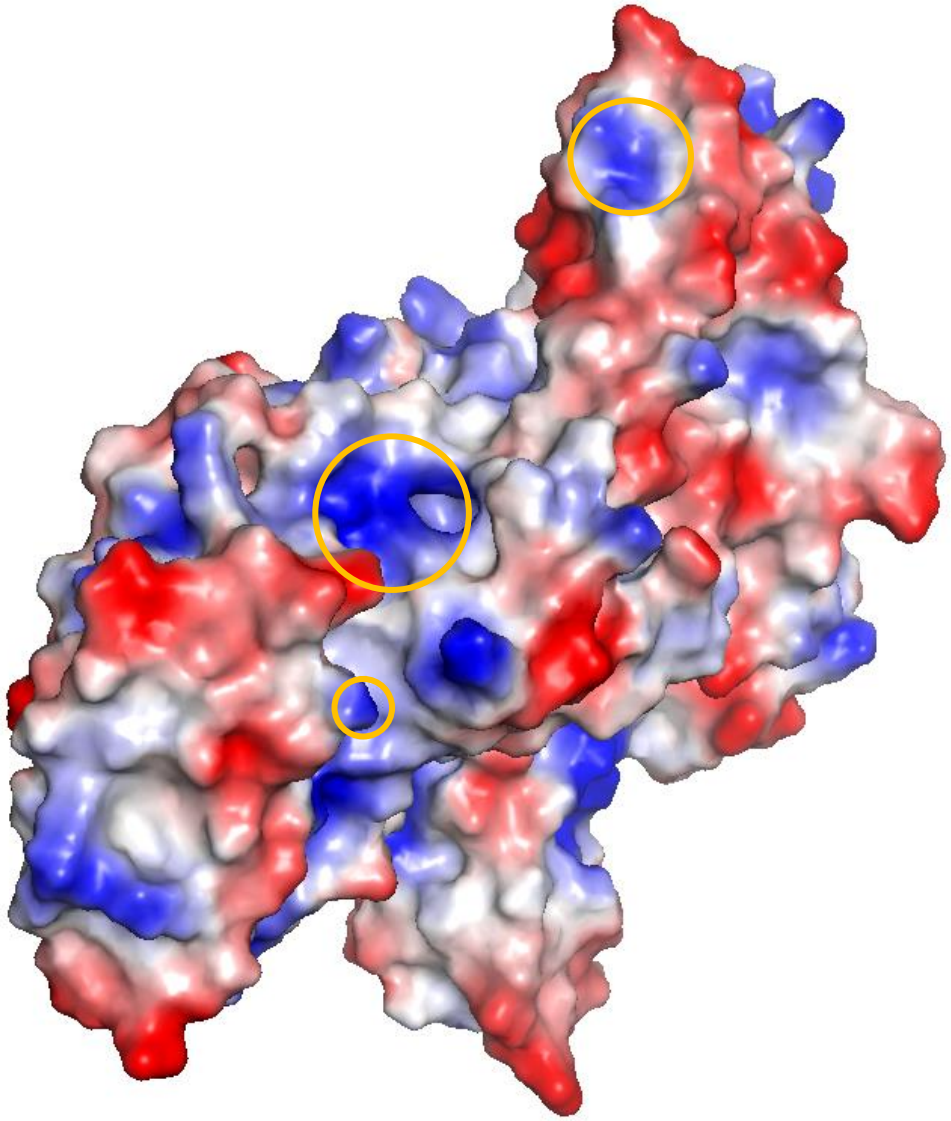
- 1 BSA
- 30 HA (Monomer)
- 160 mM Ionic Strength
- pH 7, 34˚C

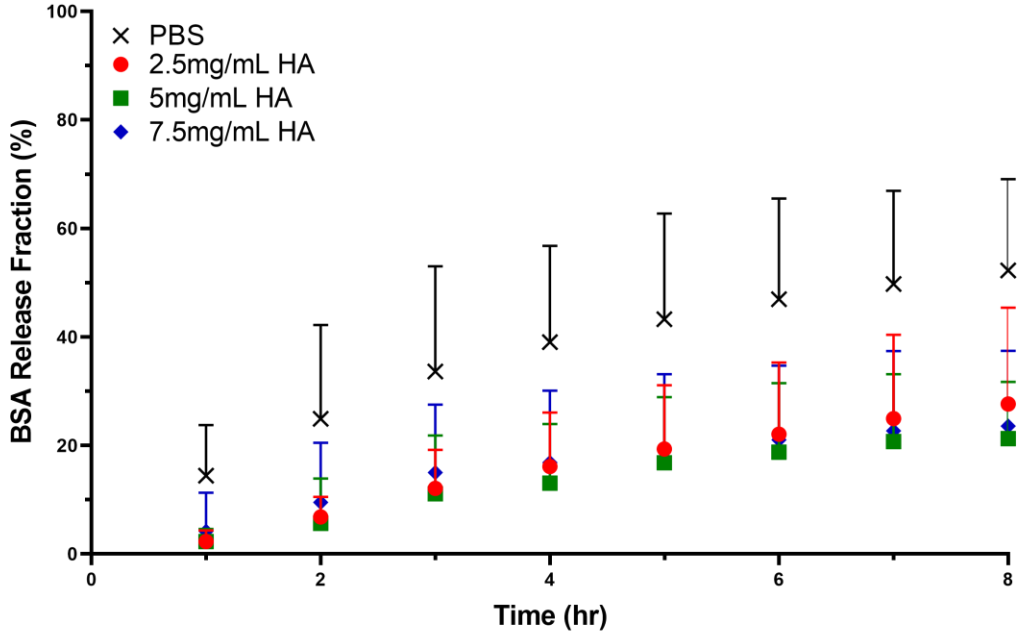
8 residues have strong interaction with HA
(2 R & 6 K)

1 MKWVTFISLL LLFSSAYSRG VFRRDTH**K**SE IAHRFKDLGE EHFKGLVLIA
51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLE**K**S HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCA**K**DDPH ACYSTVFD**K**L
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IV**R**YT**RK**VPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQL**K**TV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA

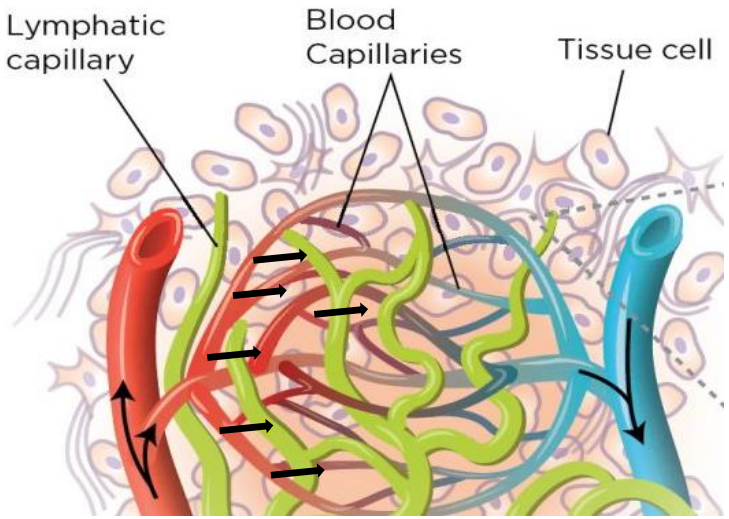
BSA – HA Electrostatic Interaction



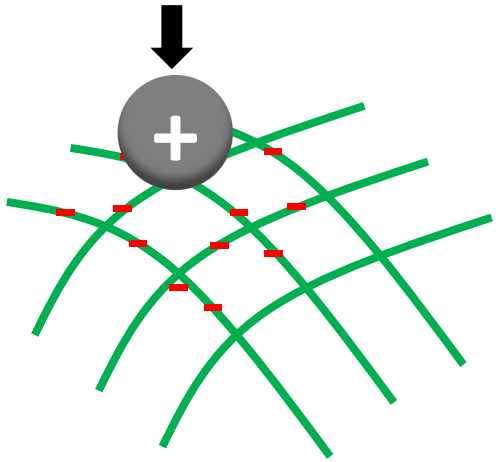


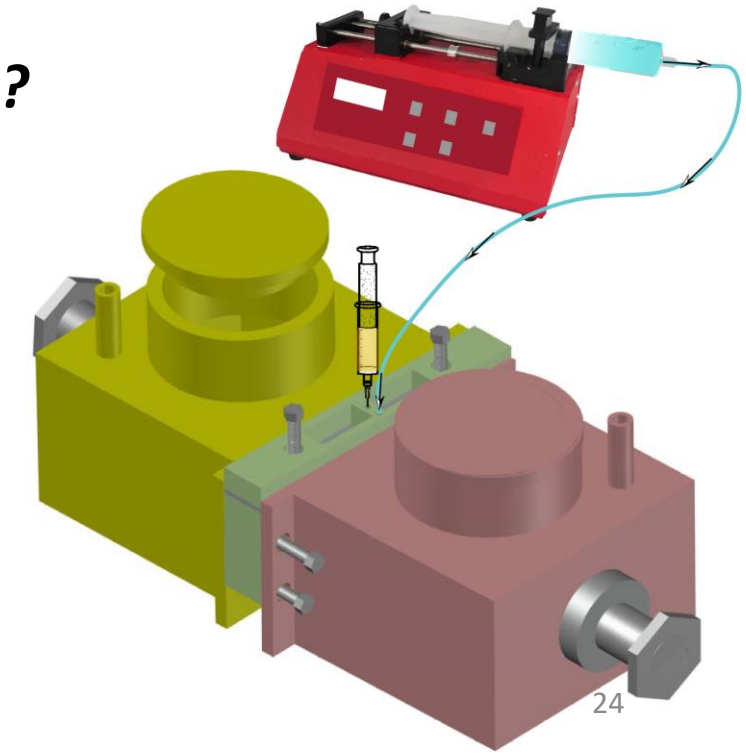


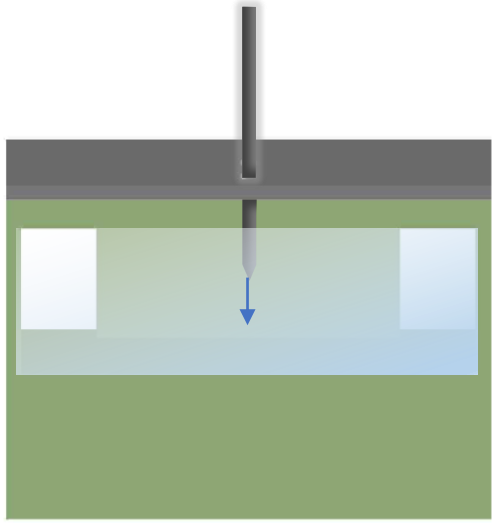
Ways to Enhance Protein Migration?



Apply Pressure to generate convection



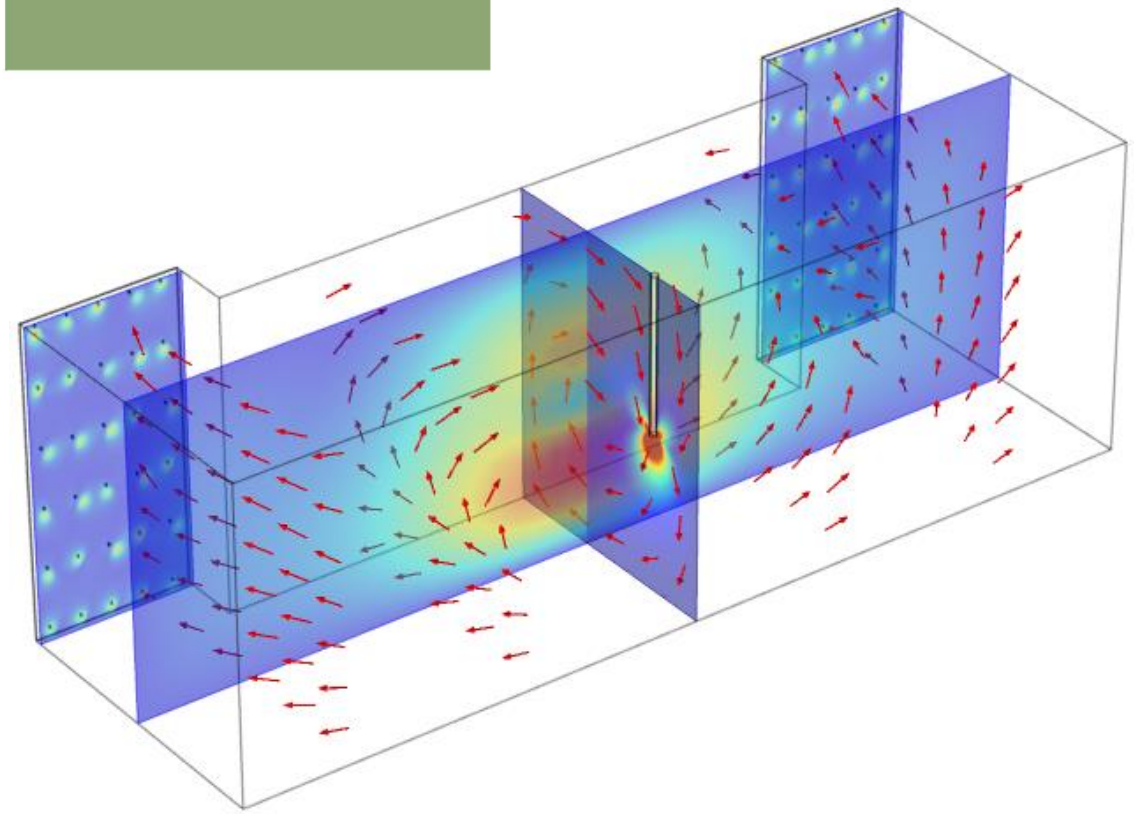




Navier-Stokes Equation

Mass Balance: $\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0$

Momentum Balance: $\rho \frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u} \cdot \nabla)\mathbf{u} = \nabla \cdot [-p\mathbf{I} + \boldsymbol{\tau}] + \mathbf{F}$



Impact of Liquid Flow on BSA Release

- BSA SKU: 15 mg / 0.5 mL
- DoE Study: 3×3=***9*** runs in triplicate

Factor 1 Liquid Flow Rate (3 levels)

0, 0.5, 1 mL/h

Factor 2 HA Concentration (3 levels)

2.5, 5, 7.5 mg/mL

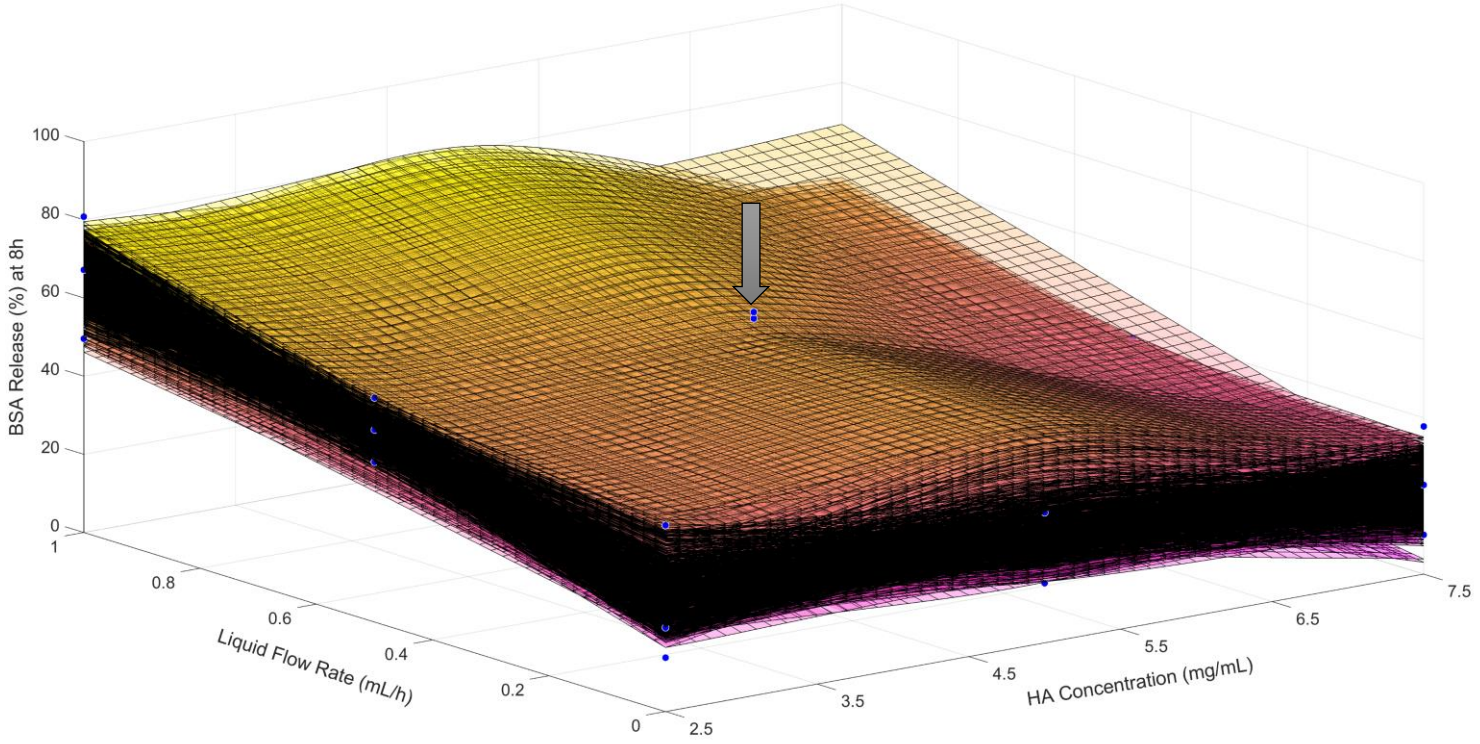
BSA Release Prediction & Modeling

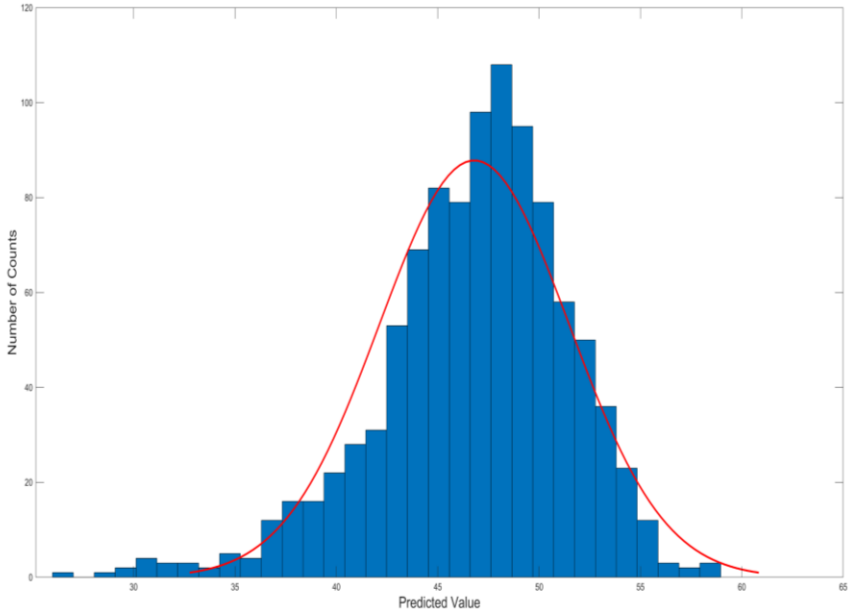
1 Dataset
27 Data Points

Bootstrap Sampling →

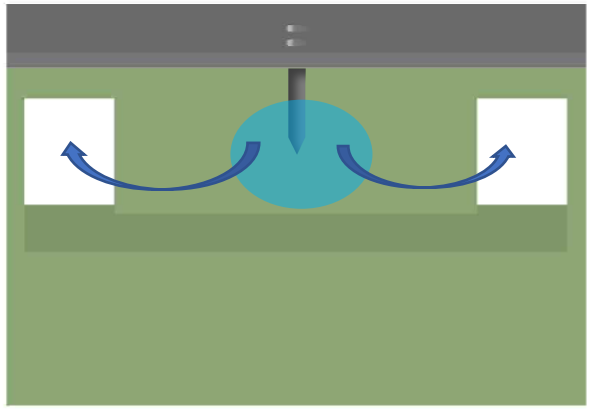
1000 Datasets
Each with 27 Data Points

HA Conc: 5 mg/mL;
Flow Rate: 0.5 mL/h;
BSA Release ~ Norm Dist.
μ: 46.8; σ: 4.68



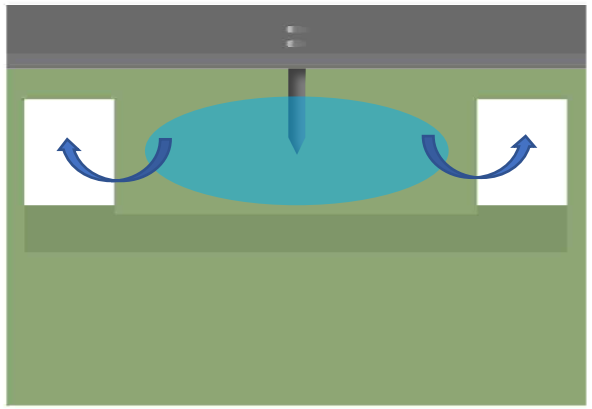


Dose/ Injection Volume -Effect on BSA (30 mg/mL) Release



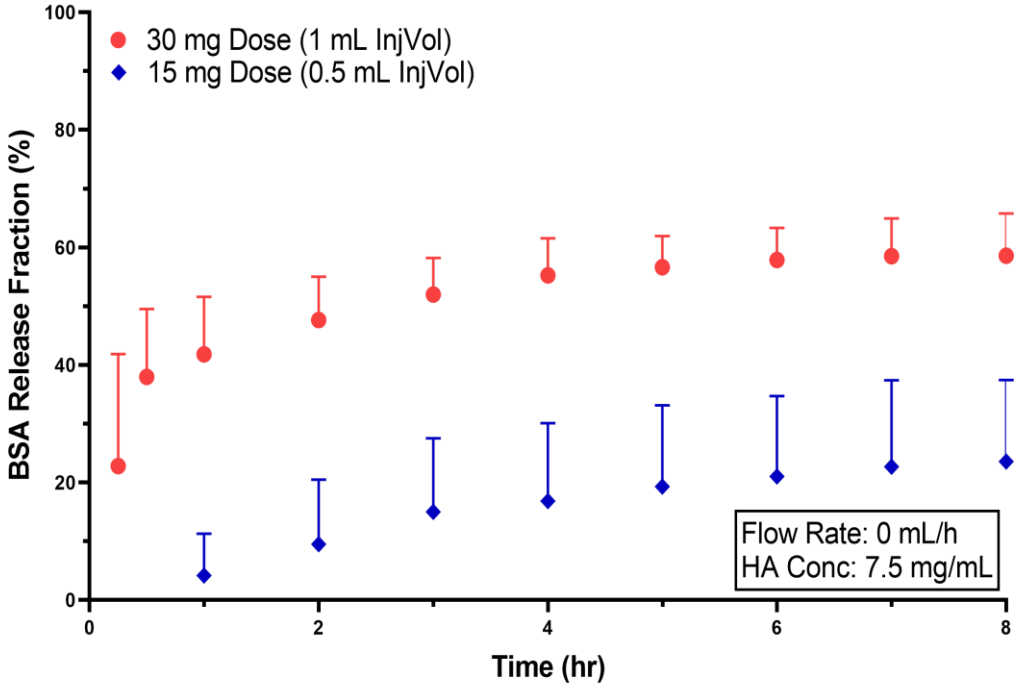
Injection Volume: 0.5 mL
(Dose: 15 mg)
Current Study

COMPLETED



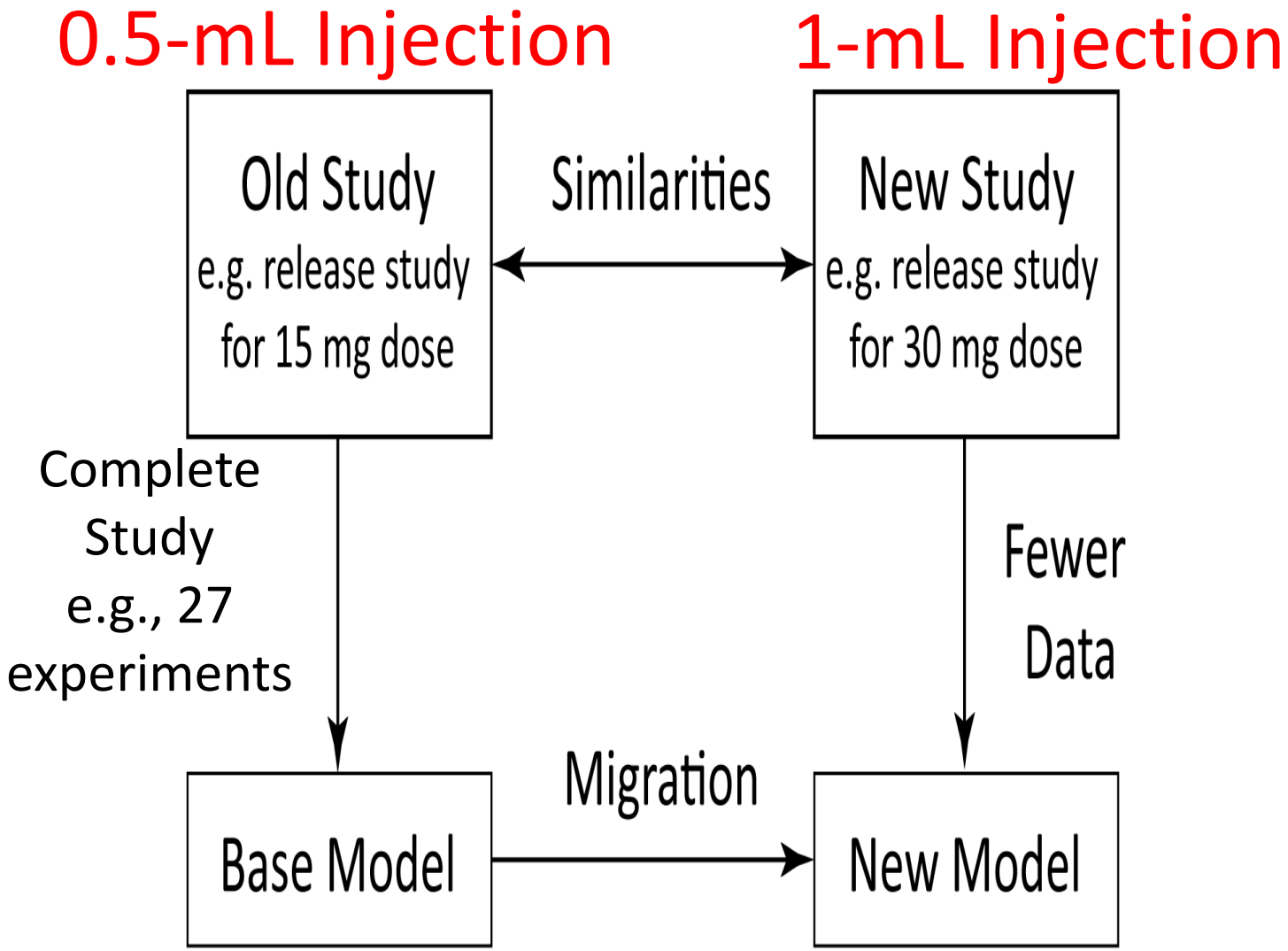
Injection Volume: 1 mL
(Dose: 30 mg)
New Study

?



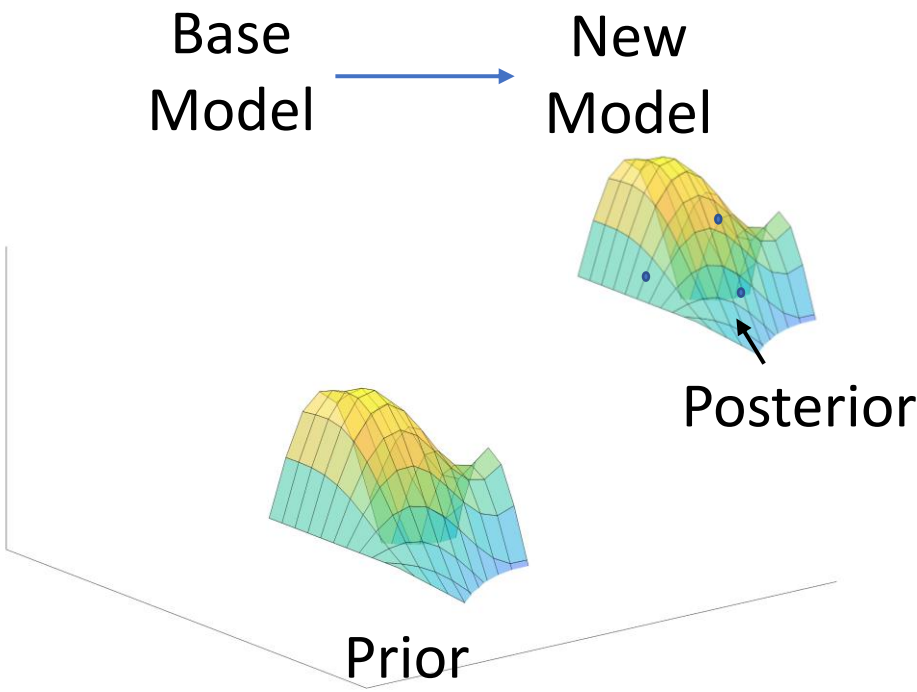
- To investigate drug release (@**30 mg dose/1 mL Injection volume**), <u>do another complete study? Can we do fewer experiments?</u>

Model Migration

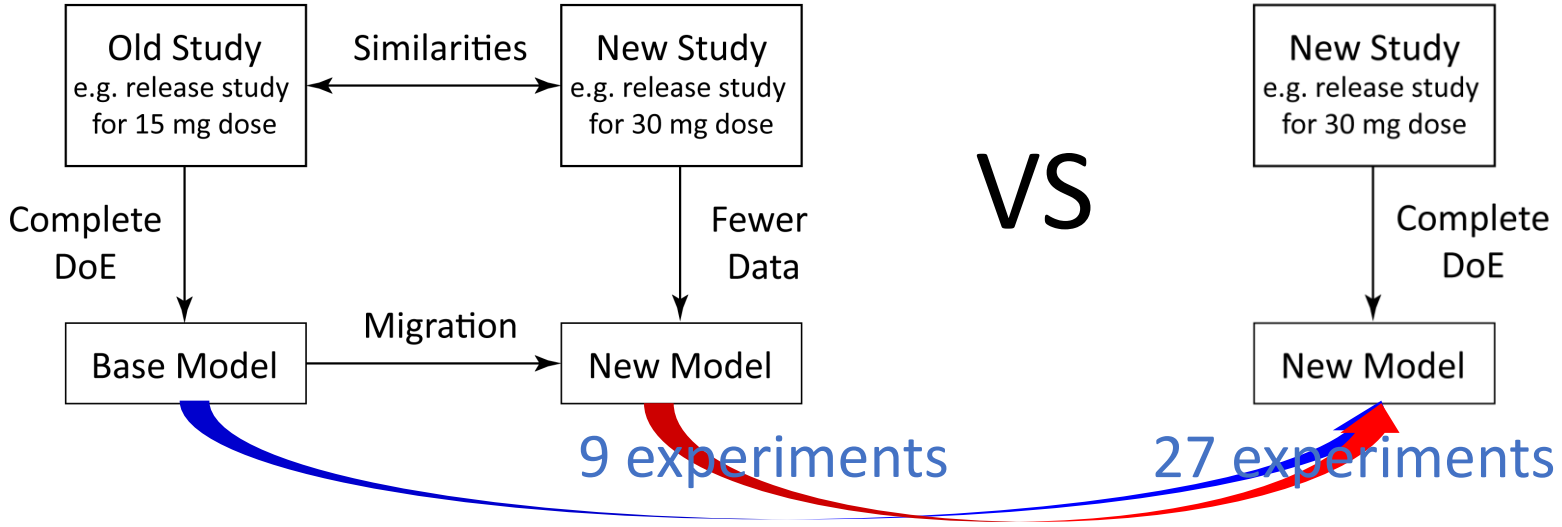


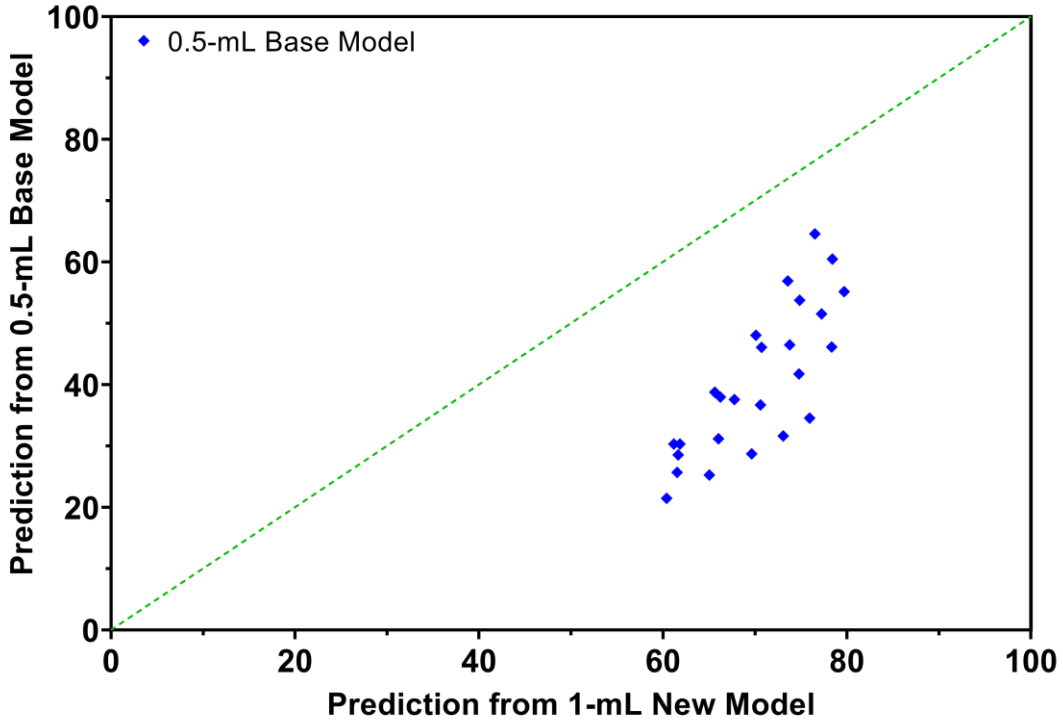
Model Migration:
Bayesian Inference Method

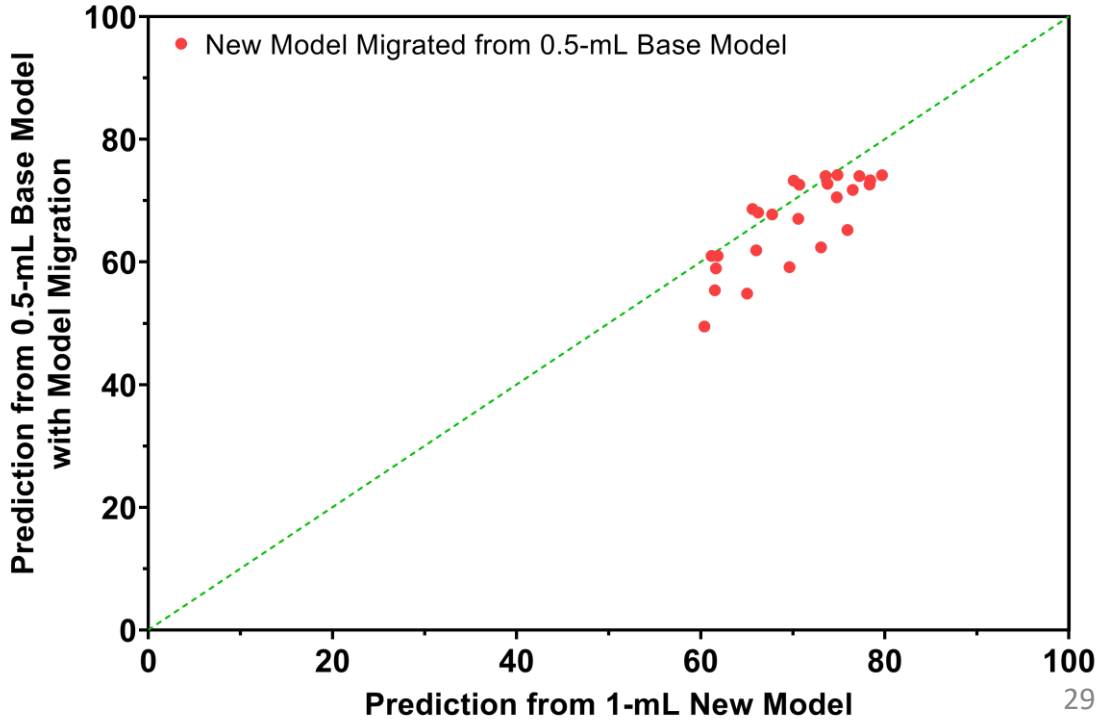
$$\mu_1 = \left(\frac{\mu_0}{\sigma_0^2} + \frac{\sum_{i=1}^{n} x_i}{\sigma_0^2}\right) \Big/ \left(\frac{1}{\sigma_0^2} + \frac{n}{\sigma_0^2}\right)$$



Model Migration







SUMMARY of Peptide & Protein Studies

Peptide (Lanreotide) Release in ESCAR

- Emulation of Lanreotide release from SubQ depots
- Impact of Excipients (HPβCD) on aggregation and release

Protein (BSA) Release in ESCAR

- Slow diffusion of BSA in HA solution
- Convection and liquid flow increases BSA release

Model Migration: Bayesian Method

- Develop model migration strategy to reduce experimental efforts related to dose/process changes and tech transfer

Thank you

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KU BioCenter
KU Nanofabrication Center