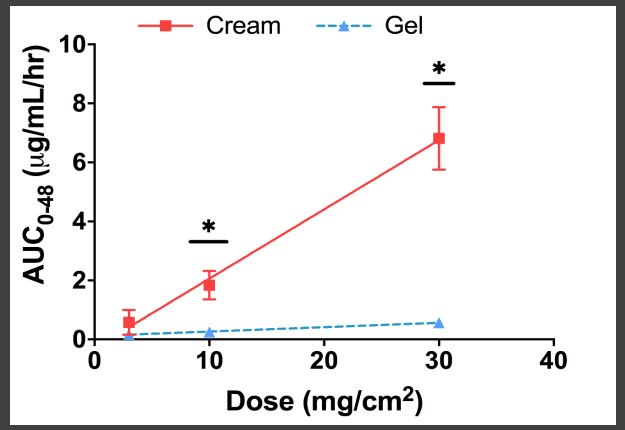
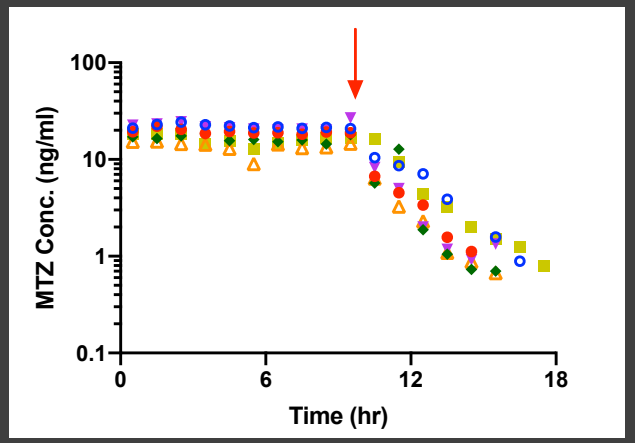
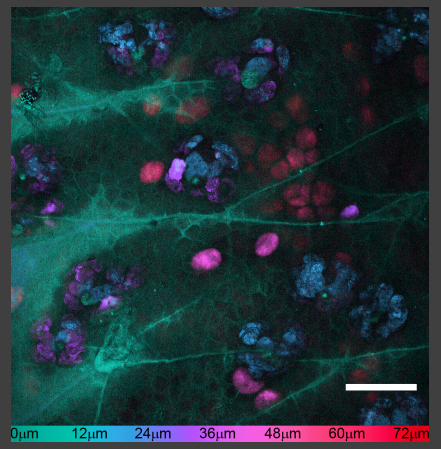
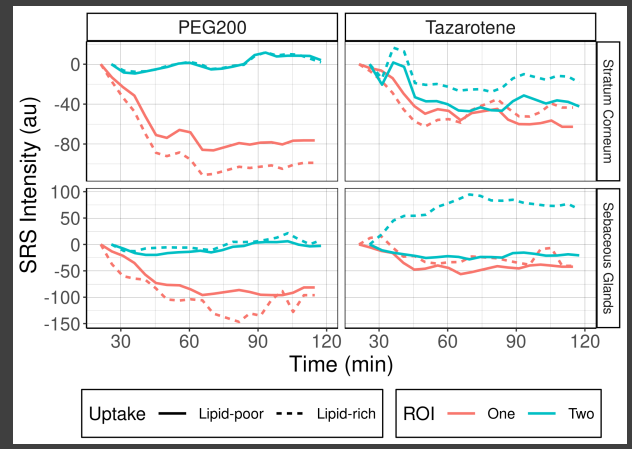
Novel Methodologies to Assess Cutaneous Bioavailability and Bioequivalence: Dermal Microdialysis and Coherent Raman Imaging









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Disclaimers

- FDA Disclaimer:
  - The views and opinions presented here represent those of the speaker and should not be considered to represent advice or guidance on behalf of the U.S. Food and Drug Administration.

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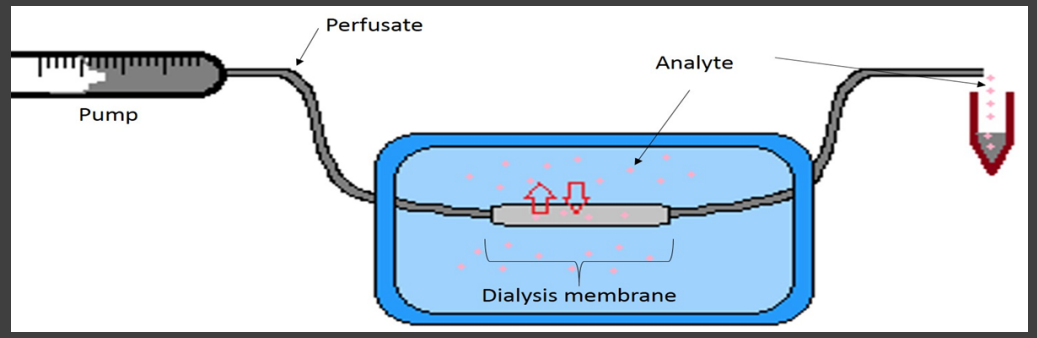
Outline

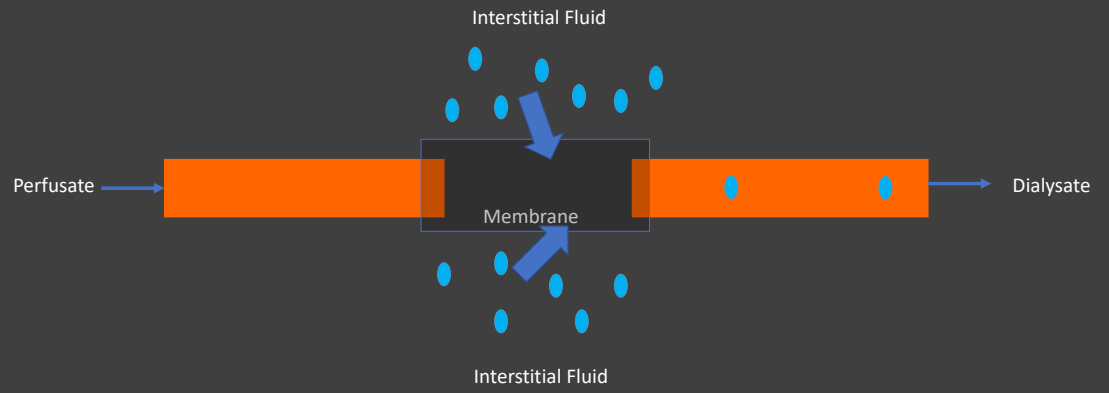
- Dermal Microdialysis (dMD)
  - Overview of Technique
  - Local Bioavailability assessment from topically applied Metronidazole products
  - Identification of flip-flop cutaneous pharmacokinetics
- Coherent Raman Scattering Imaging (CRI)
  - Overview of Technique
  - Proof of concept investigation with Ruxolitinib
  - Accessibility of fingerprint region signatures
    - Allows quantification of many topical APIs

Dermal Microdialysis (dMD)

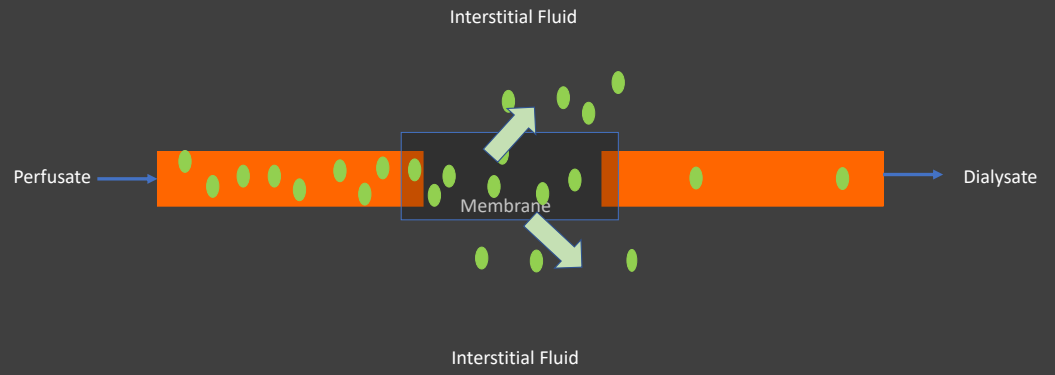
- MD is an in vivo, minimally invasive technique, that allows sampling of **unbound** molecules in the dermis and subcutaneous tissues



Microdialysis (*Gain*) Principle



Retrodialysis (*Loss*) Principle

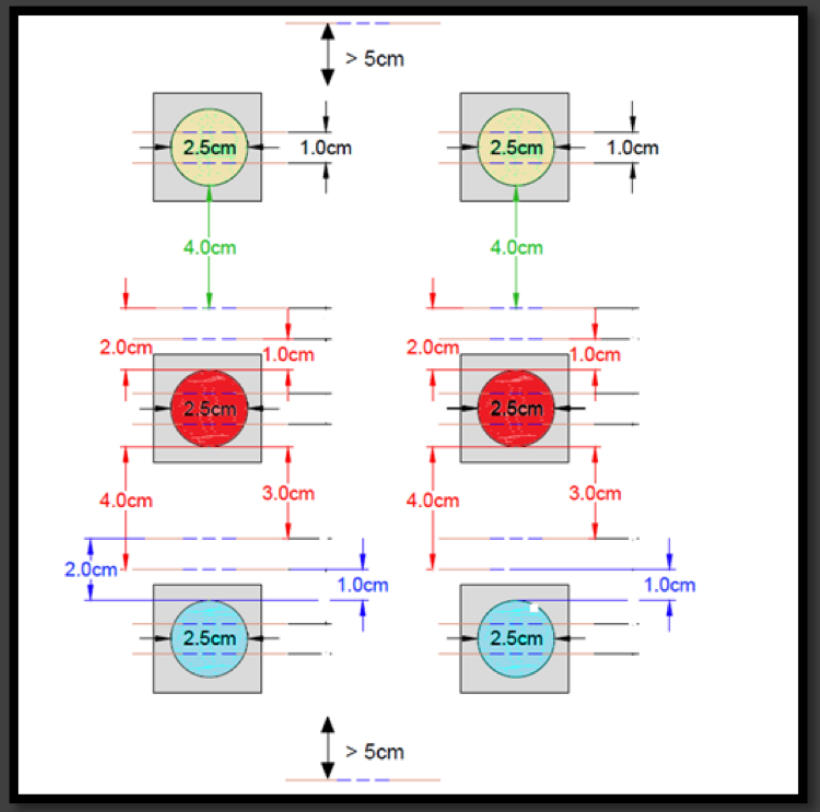


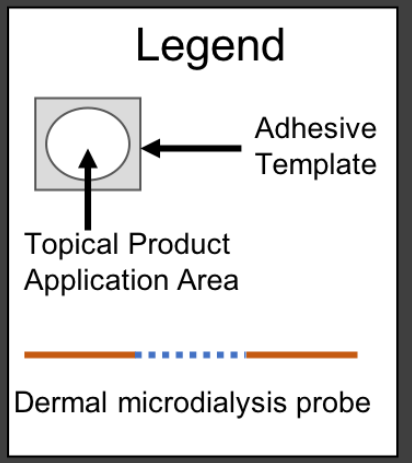
Local BA of Metronidazole (MTZ) Study

- Are dMD probes sensitive to changes in the local dermal environment?
- What is the minimum distance between application sites for no cross talk?

Formulations

- Generic Gel: MTZ topical gel, 0.75% from Tolmar
- Generic Cream: MTZ topical cream, 0.75% from Fougera Pharma





Dose:

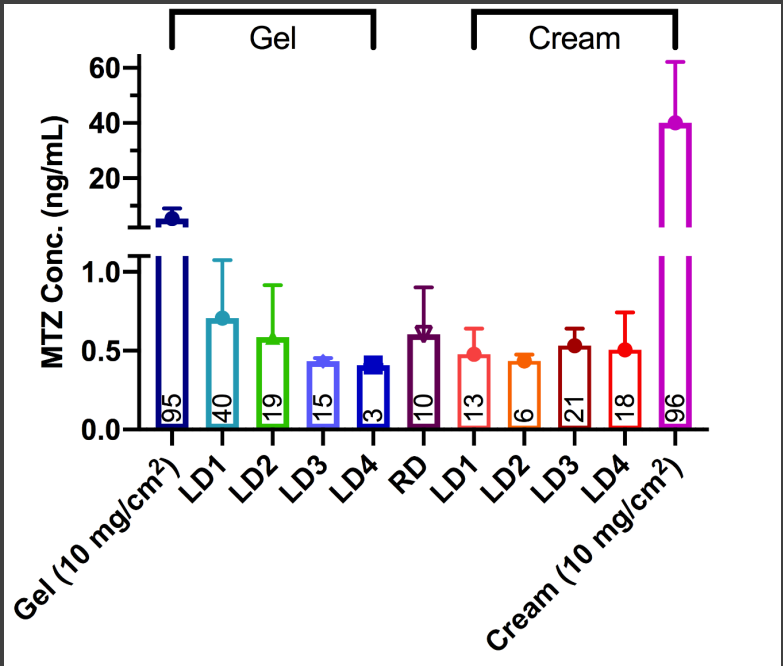
3 mg/cm^2

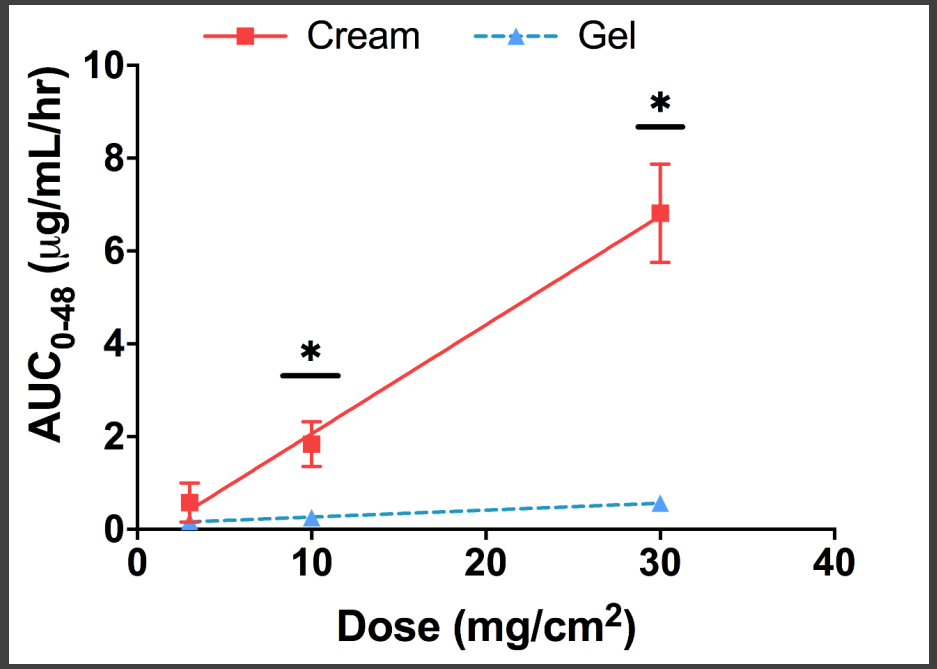
10 mg/cm^2

30 mg/cm^2

Kuzma, Benjamin A., et al. *European Journal of Pharmaceutical Sciences* 159 (2021): 105741.

Negligible Lateral Diffusion and Linear Dose Response





- Theoretical distances from clinical dose (10 mg/cm^2)

✓ Concentrations detected in LD probe were similar to those in the RD

✓ No *significant* trend moving away from application site

✓ No systemic redistributions – Contributions in LD probe attributed to MTZ impurity in D_3-MTZ

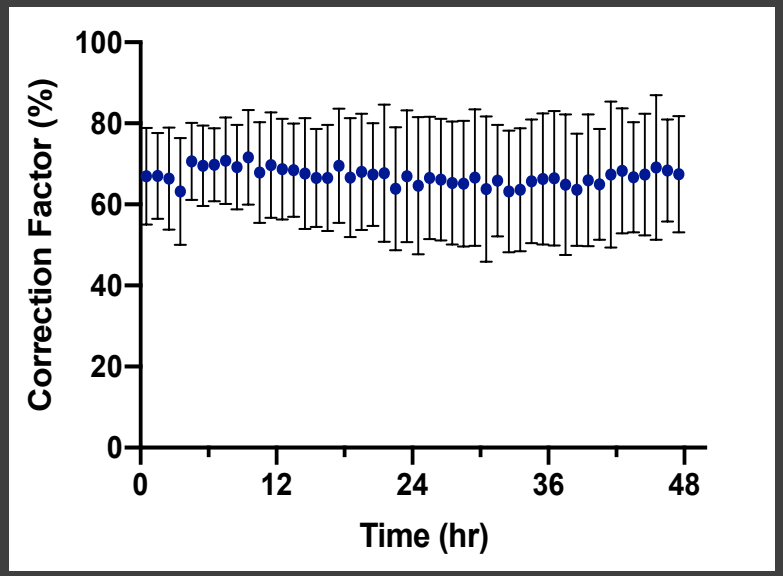
✓ Significant exposure difference between gel and cream products at 10 mg/cm^2 ($p = 0.009$) and 30 mg/cm^2 dosing ($p = 0.0005$)

✓ Significant difference between slopes ($p = 0.0042$) with linear ($R^2>0.99$) and proportional increase in $AUC_{0\text{-}48}$ with dose

✓ dMD able to discriminate between formulations and sensitive to different local bioavailabilities

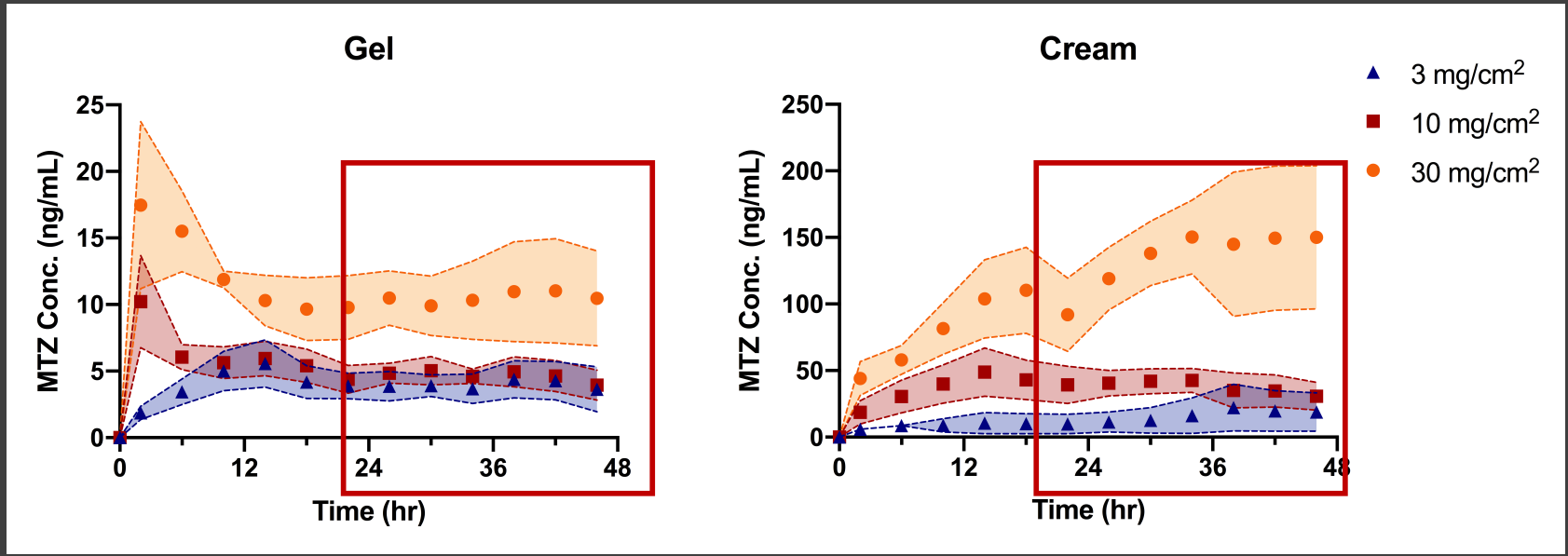
Kuzma, Benjamin A., et al. *European Journal of Pharmaceutical Sciences* 159 (2021): 105741.

dMD Probe Stable For 48-hr Yet No Terminal Phase



- Concentrations begin to level off or increase after 24-hr
- Unreliable estimate of Cmax

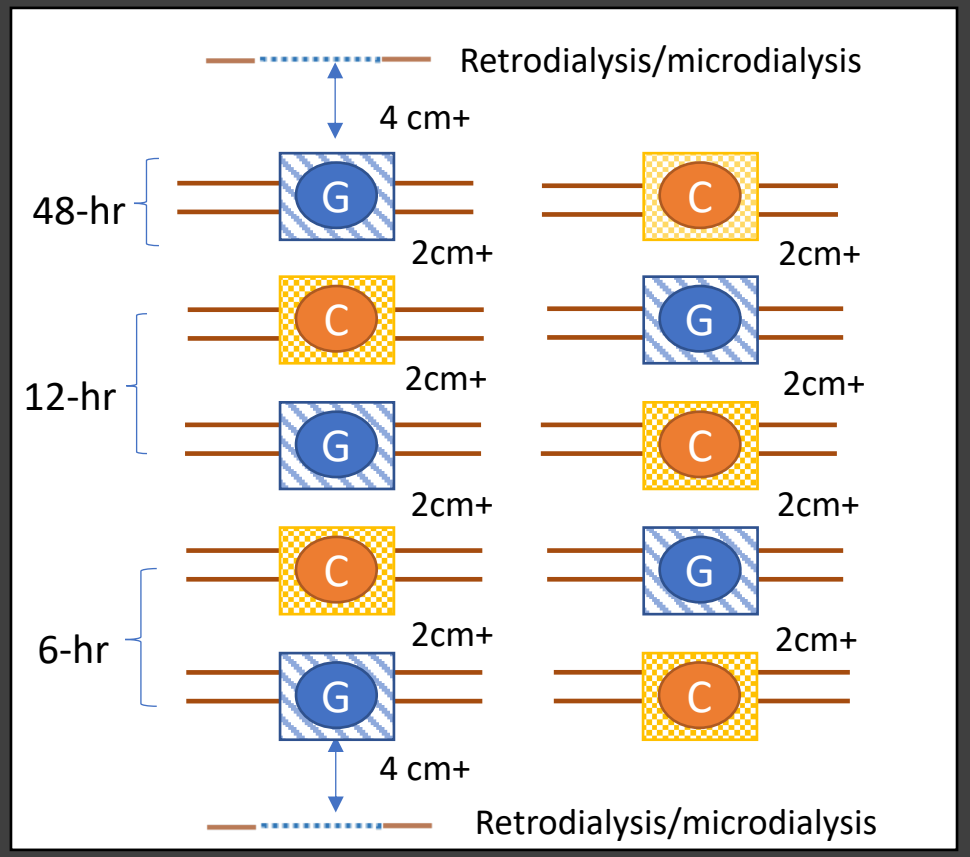
- Ability to correct concentrations by correction factor can account for random fluctuations
- No probe fouling or deterioration over the course of the study duration



Kuzma, Benjamin A., et al. *European Journal of Pharmaceutical Sciences* 159 (2021): 105741.

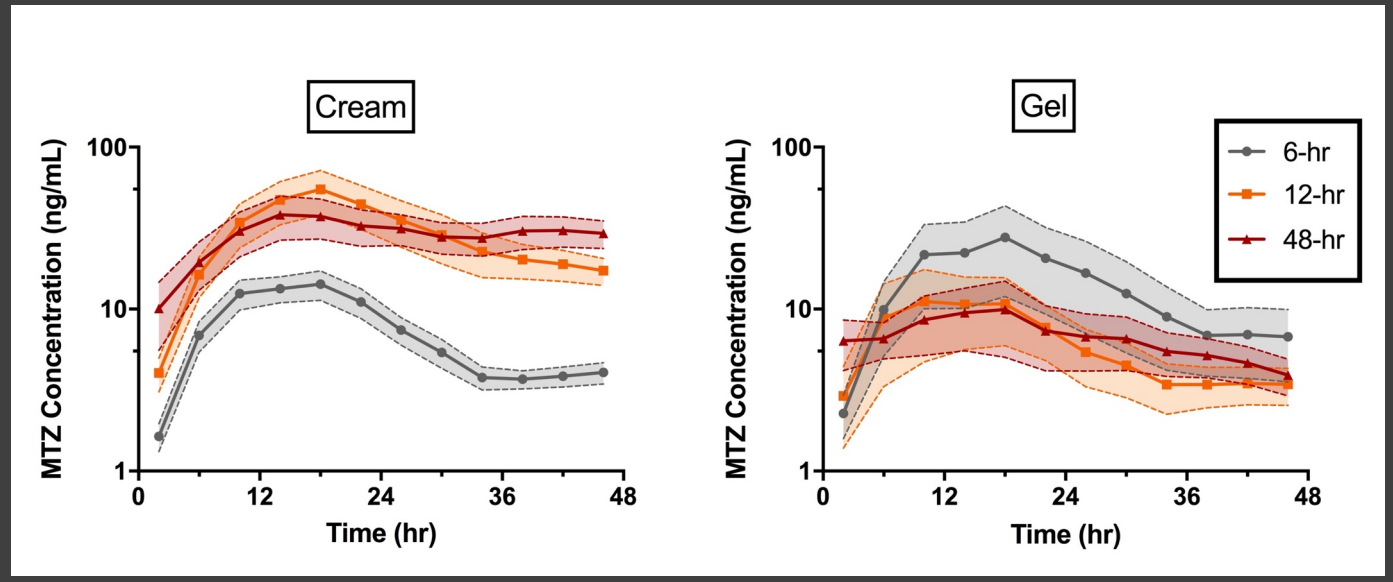
Dose Duration Effect on dBA Study

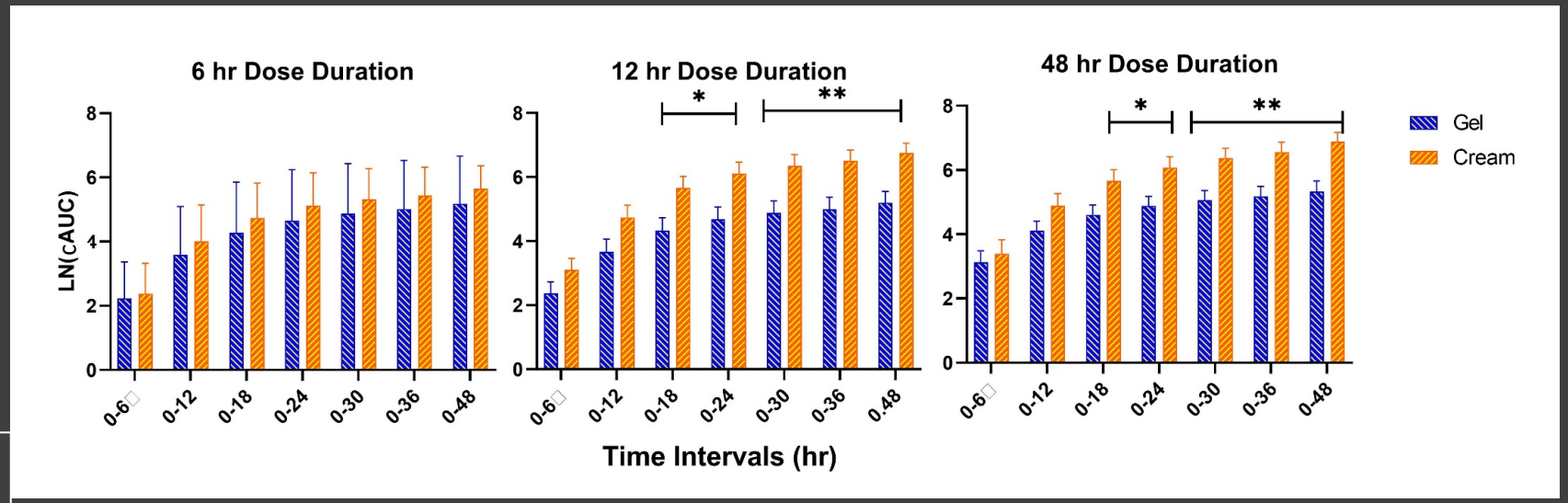
- Does the dose duration impact dermal BA?
  - Formulations applied for:
    - 6-hr
    - 12-hr
    - 48-hr (no removal)
- Can we estimate the dermal disposition of metronidazole using dMD?



Kuzma, Benjamin A., et al. *European Journal of Pharmaceutics and Biopharmaceutics* 175 (2022): 43-52.

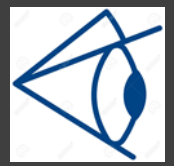
Adequate Characterization of Dermal PK

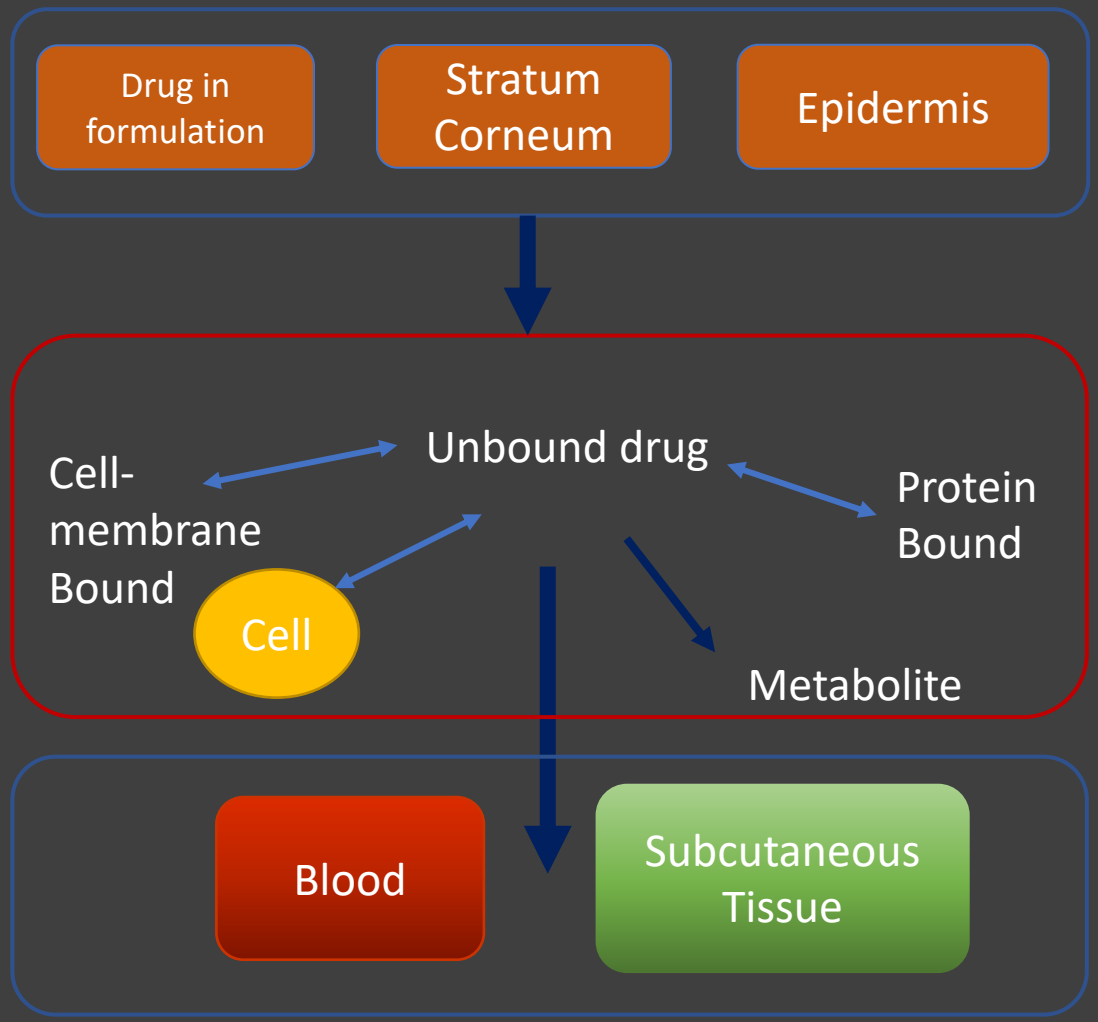




Kuzma, Benjamin A., et al. *European Journal of Pharmaceutics and Biopharmaceutics* 175 (2022): 43-52.

The observed dermal concentration profile results from:



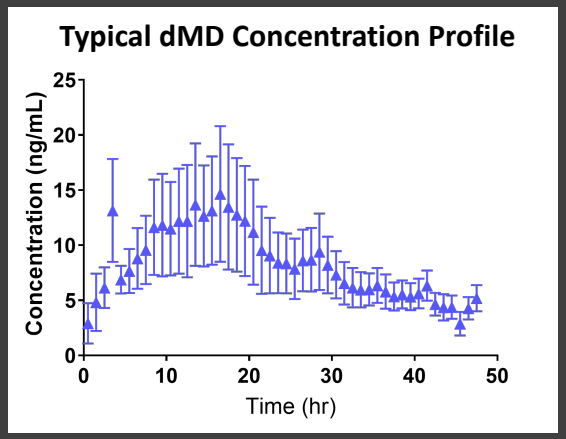


Absorption (f_t)

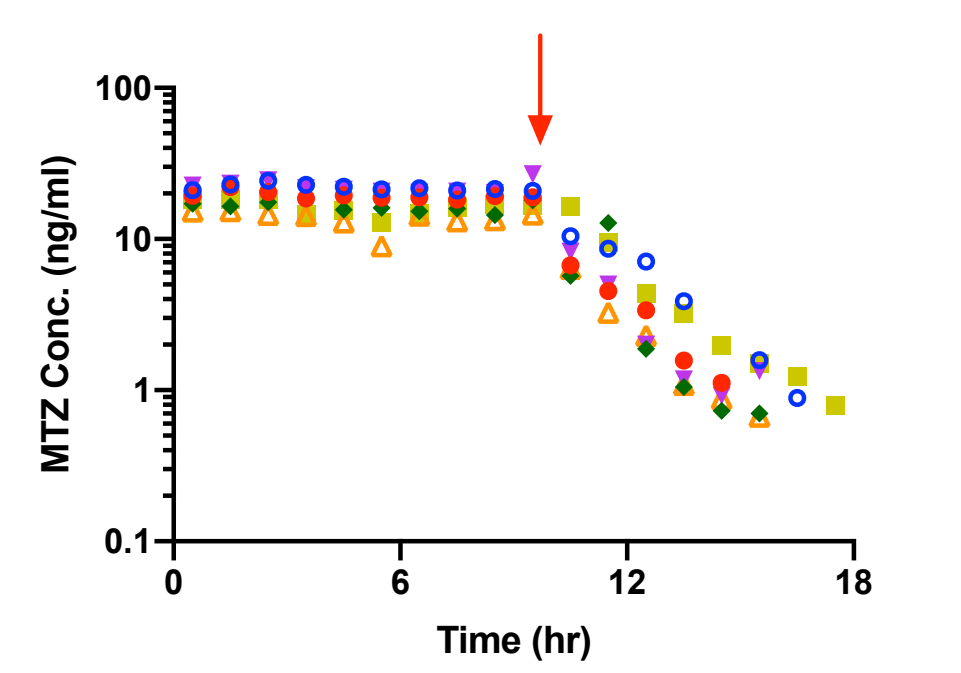
Distribution within dermis

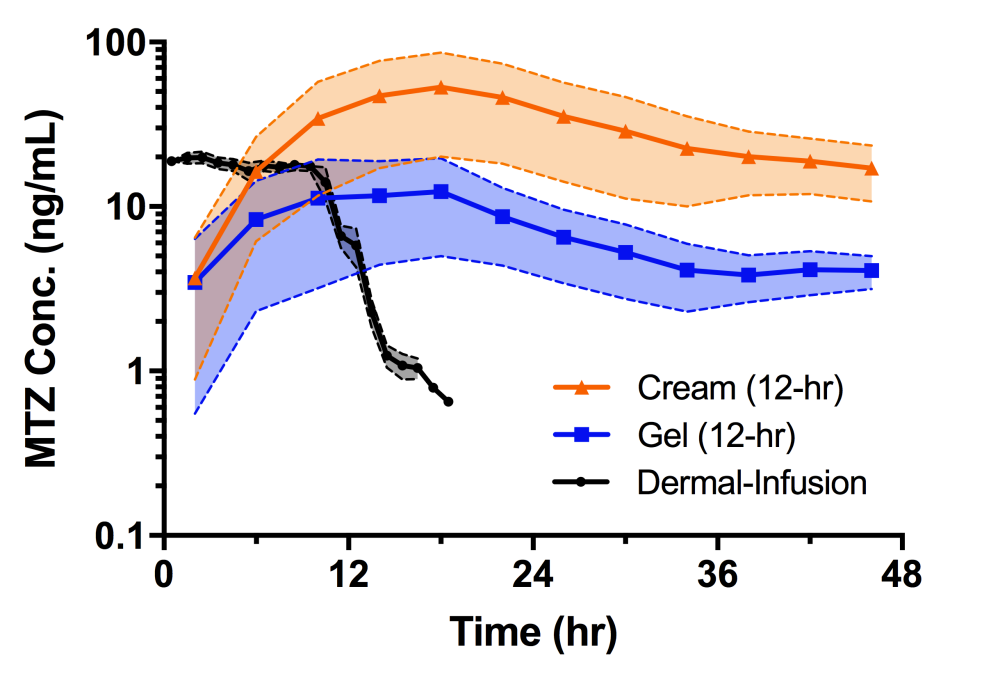
Elimination

Disposition (g_t)



MTZ Dermal Disposition



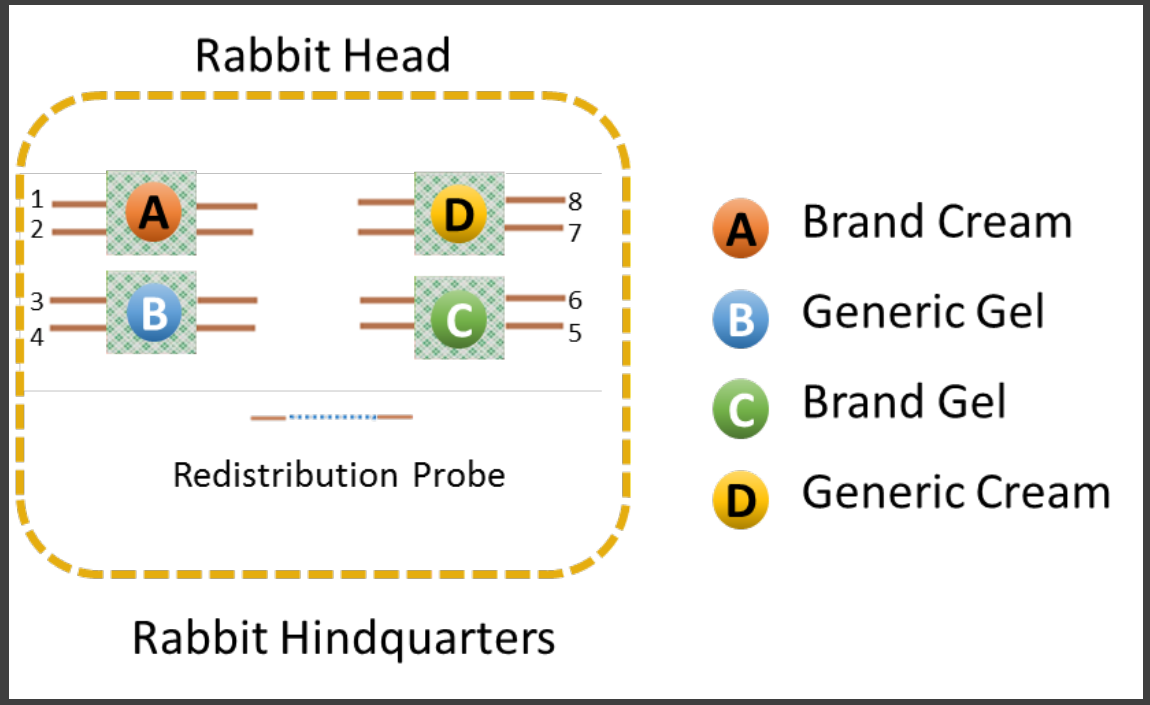


- The terminal phase after topical administration is actually the **absorption** half-life for topically applied metronidazole
- Using systemic half-life to estimate absorption function will likely underestimate the dermal exposure

Kuzma B.A., et al. (2019) Estimation Of In Vivo Skin Permeation (Flux) And Cumulative Amount Input of Metronidazole Formulations in Mini-pigs' Dermis GRS/GRC – Skin Barrier Function of Mammalian Skin, Waterville Valley, N.H. **2019**.

Rabbit MTZ BE study

- Can dMD be used to identify differences in local BA between:
  - Two types of vehicles (cream/gel)
  - Test vs. reference in same vehicle

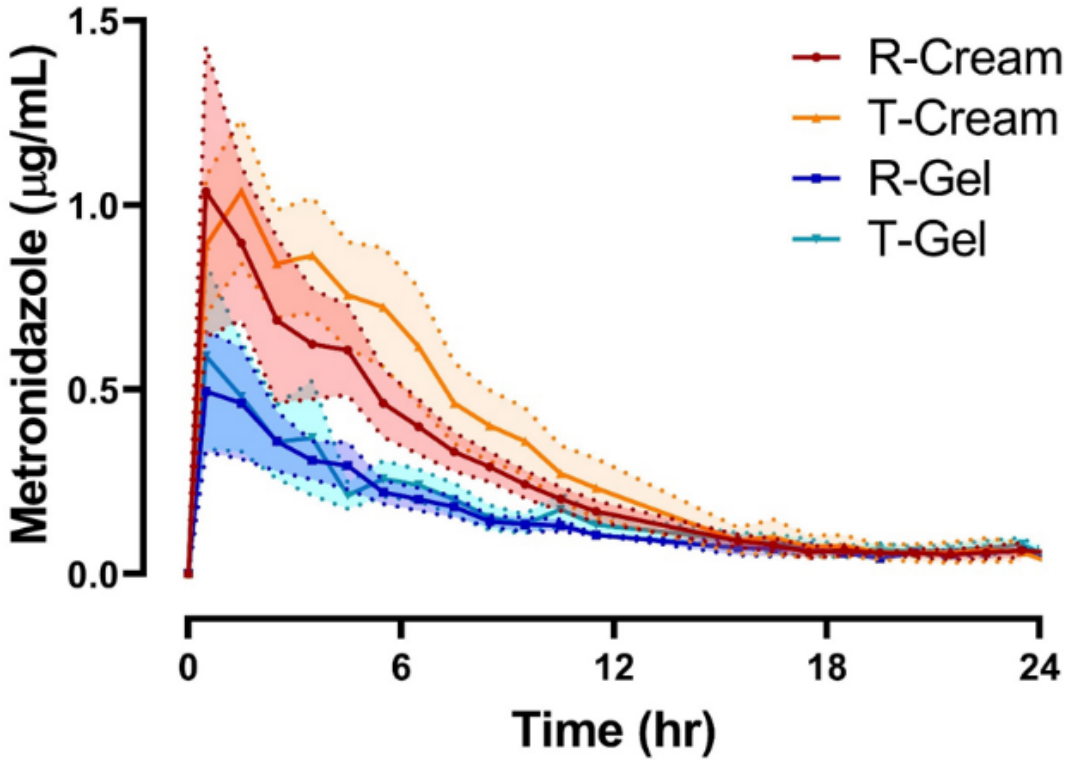


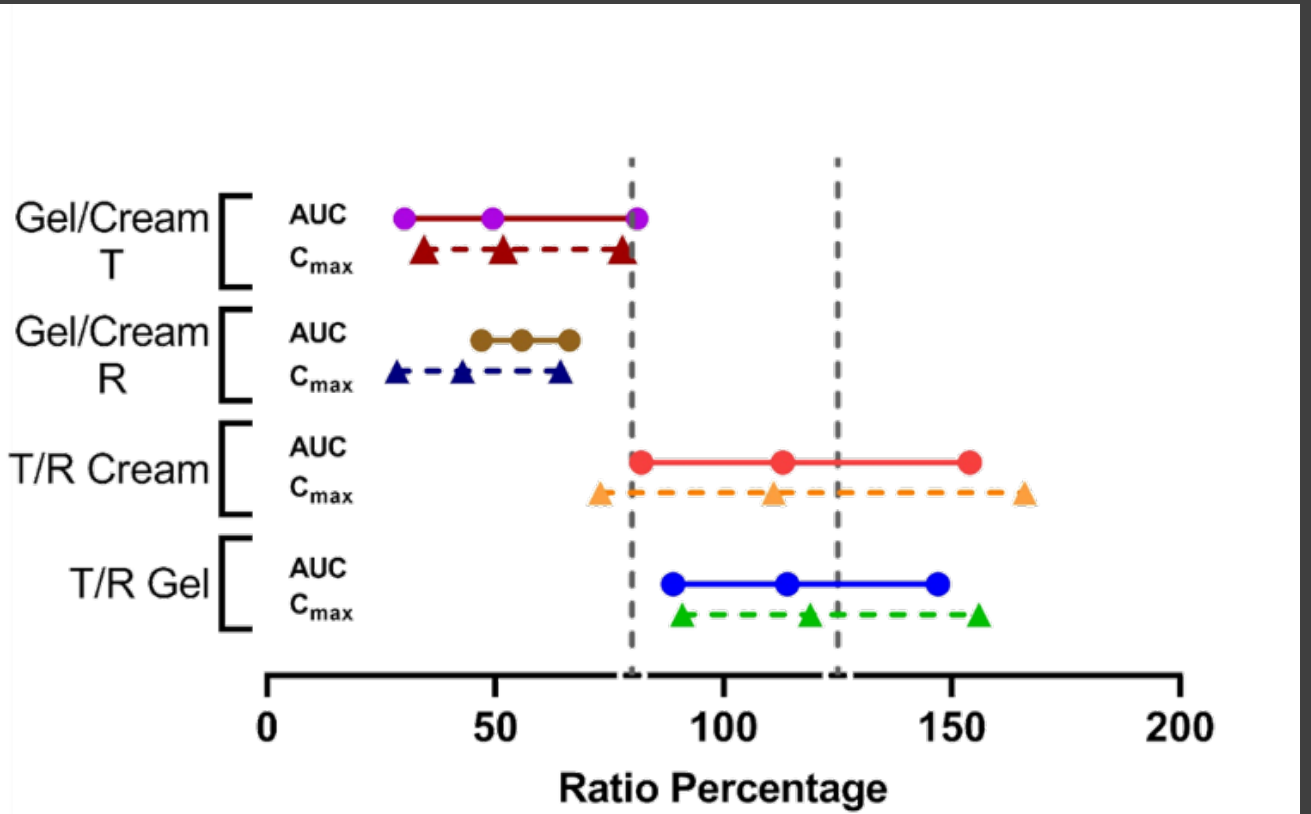
Formulations

- Brand Gel: MetroGel® topical gel, 0.75% from Prasco Labs
- Generic Gel: MTZ topical gel, 0.75% from Tolmar
- Brand Cream: MetroCream® topical cream, 0.75% from Galderma Laboratories
- Generic Cream: MTZ topical cream, 0.75% from Fougera Pharma

Senemar S, et al. (2019) Evaluating the Bioequivalence of Topical Dermatological Drug Products Containing Metronidazole Using Dermal Microdialysis: Preliminary Studies in Rabbits. AAPS 2019

Adequate BA and BE assessment

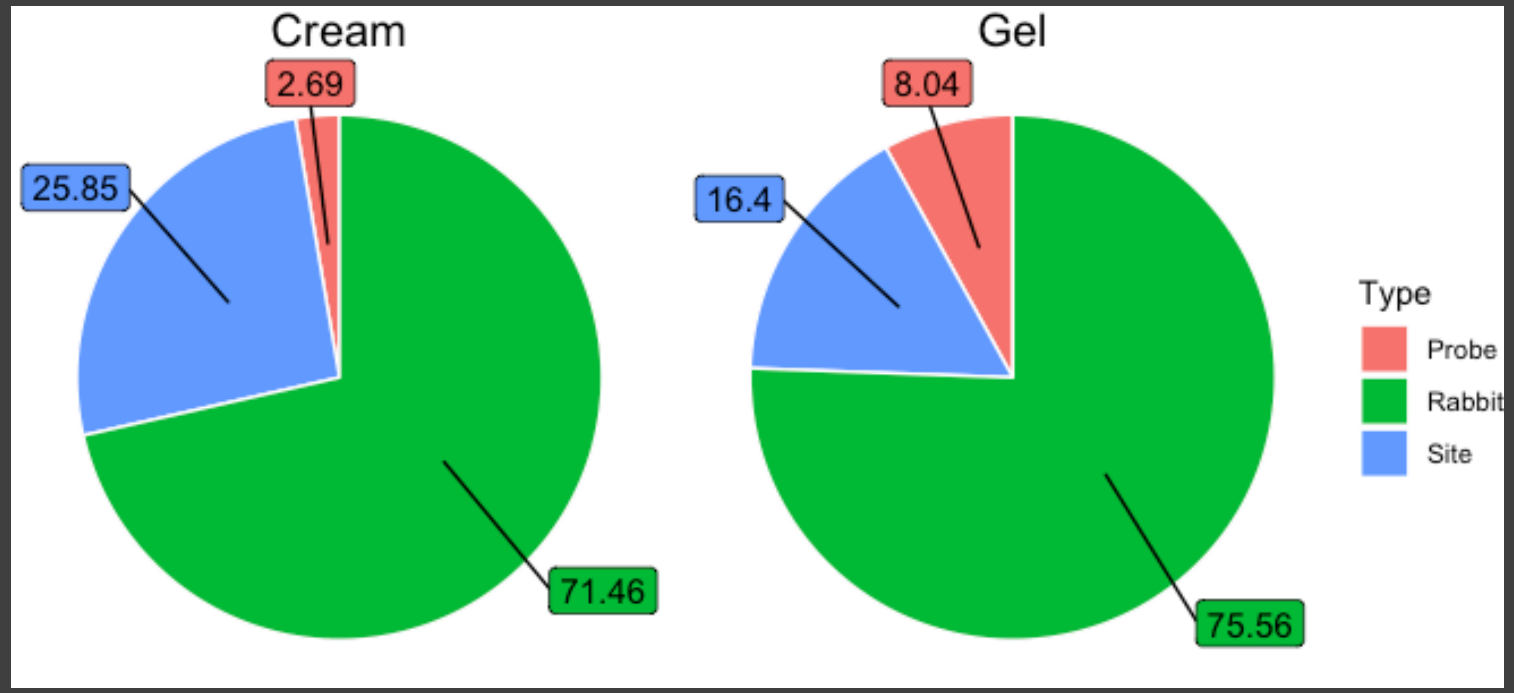




Senemar S, et al. (2019) Evaluating the Bioequivalence of Topical Dermatological Drug Products Containing Metronidazole Using Dermal Microdialysis: Preliminary Studies in Rabbits. AAPS 2019

What components contribute to the variability?

- Total CV of log(AUC_{0-24}) between 42-55%



Senemar *et al.* (Metronidazole)
Inter-subject variability – 71-76 %
Intra-subject variability – 24-28 %

Senemar S, et al. (2019) Evaluating the Bioequivalence of Topical Dermatological Drug Products Containing Metronidazole Using Dermal Microdialysis: Preliminary Studies in Rabbits. AAPS 2019

Benfeldt *et al.* (Lidocaine)
Inter-subject variability – 61 %
Intra-subject variability – 39 %

Benfeldt et al., J Invest Dermatol. 2007 Jan;127(1):170-8. Epub 2006 Jul 27

Ortiz *et al.* (Metronidazole)
Inter-subject variability – 116-223%*
Intra-subject variability – 30-39%*

Ortiz, P. García, et al. *Skin pharmacology and physiology* 24.1 (2011): 44-53.

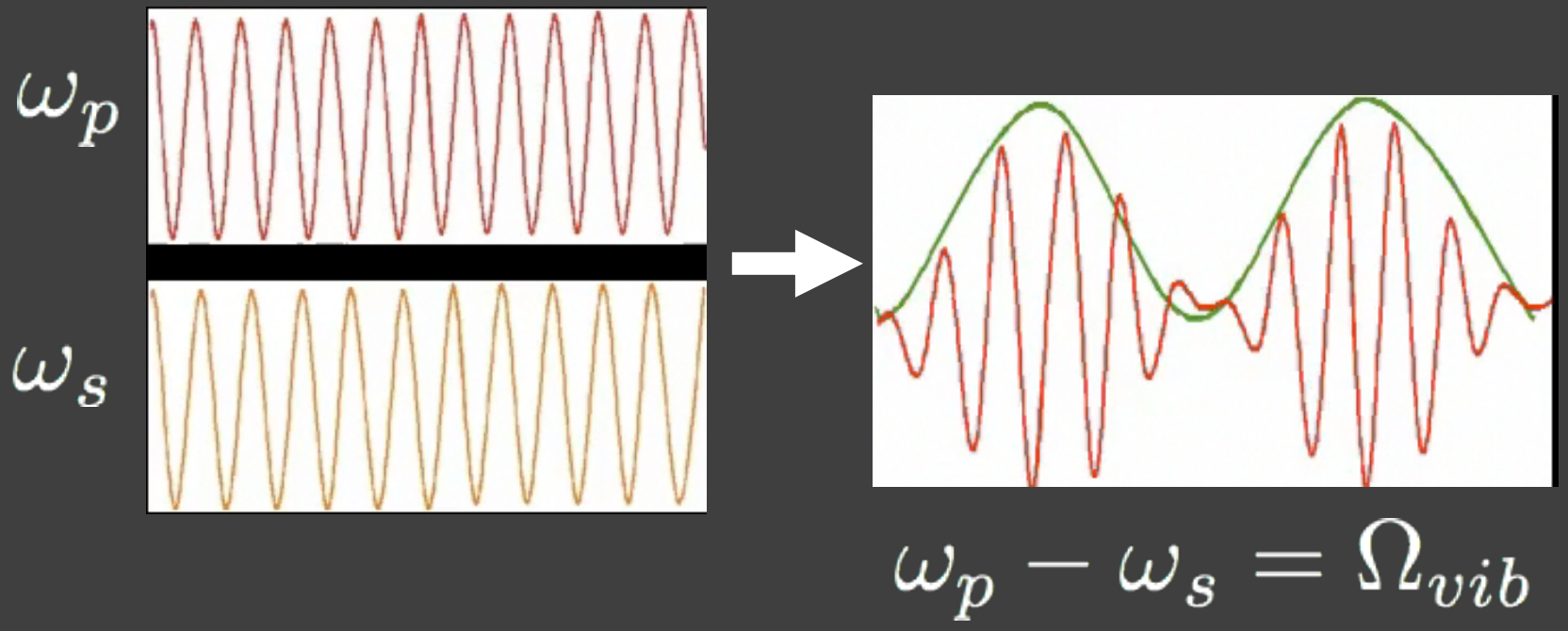
Coherent Raman Scattering Imaging (CRI)

CRI microscopy principles

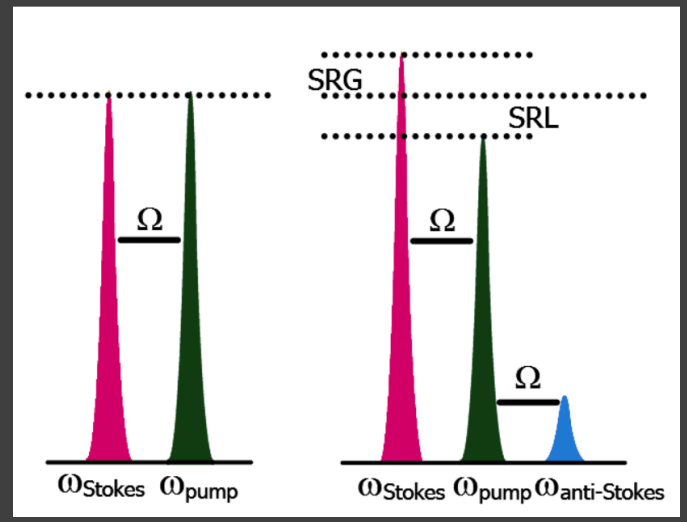
Imaging based on intrinsic vibrational contrast

Two Colors: ω_p "Pump"

ω_s "Stokes"



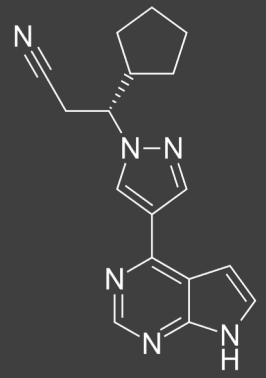
$$\omega_p - \omega_s = \Omega_{vib}$$



Pence, I. J., and Evans, C.L. *Analyst* (2021)

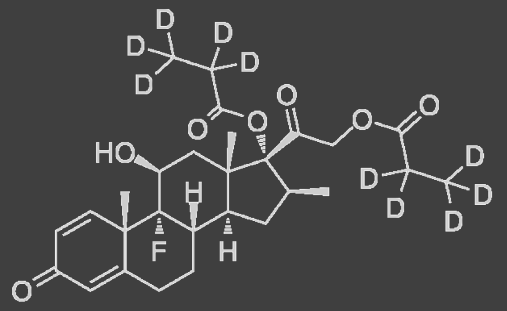
How do formulations impact drug disposition?

Ruxolitinib (100 mM)



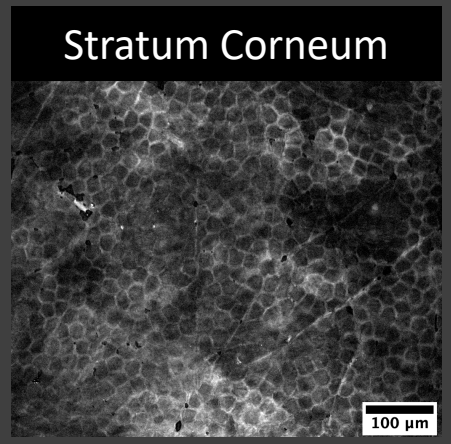
Nitrile Stretch: 2250 cm^{-1}
100% resonant signal

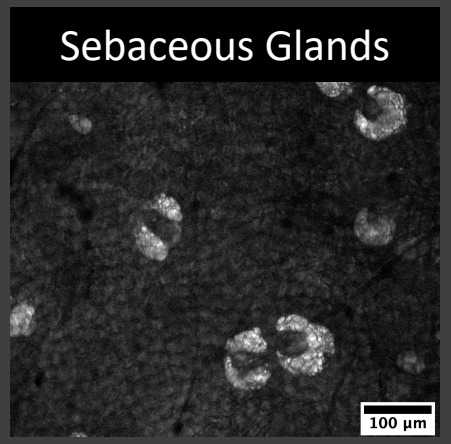
D-Betamethasone (100 mM)

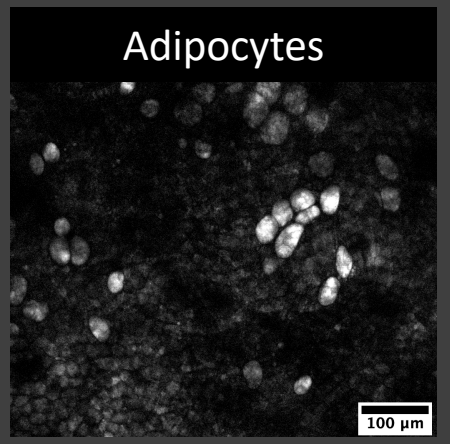


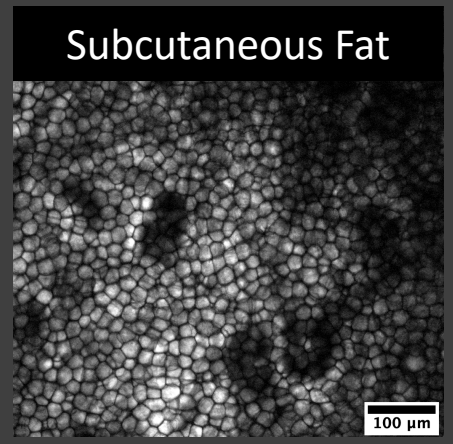
CD2/CD3 Stretch: 2200 cm^{-1}
100% resonant signal

- Gel formulation
- Transcutol formulation
- Fresh Ex vivo nude mouse ear







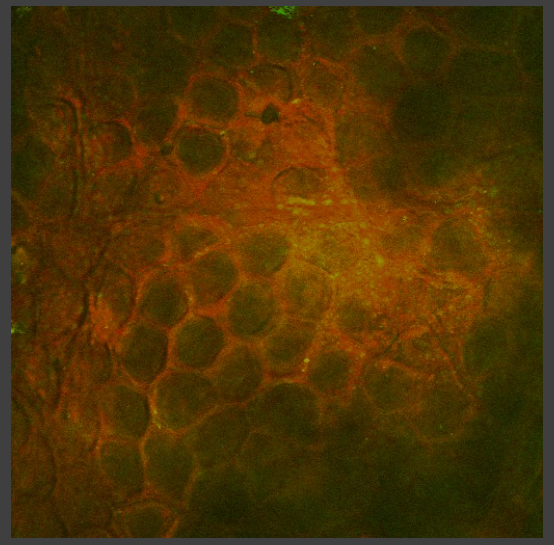


Kuzma, B.A. et al. *Journal of Visualized Experiments* (2021)

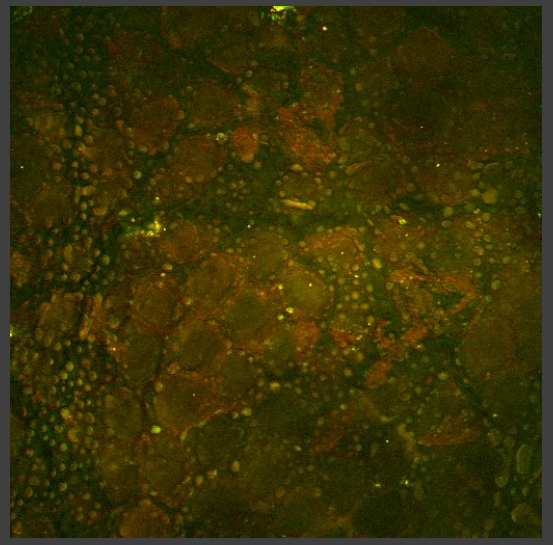
Rux Uptake in the Stratum Corneum

Red: Lipid 2845 cm^{-1}

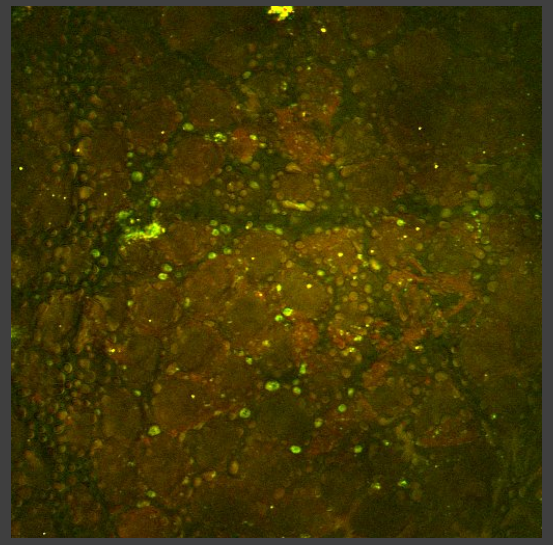
Green: Nitrile 2250 cm^{-1}



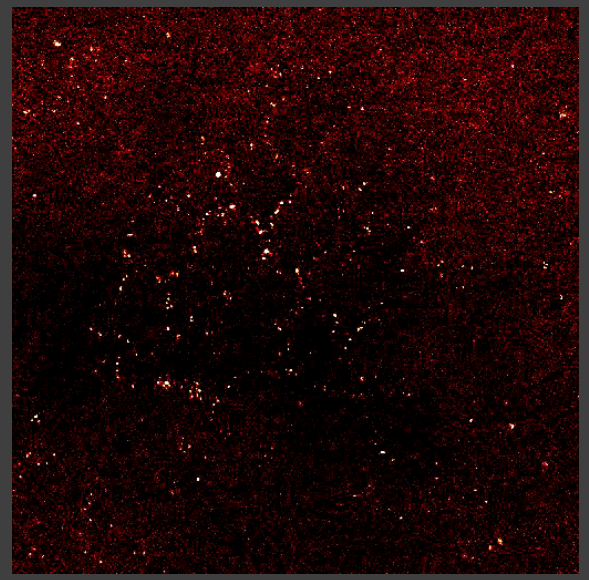
15 min



70 min



120 min

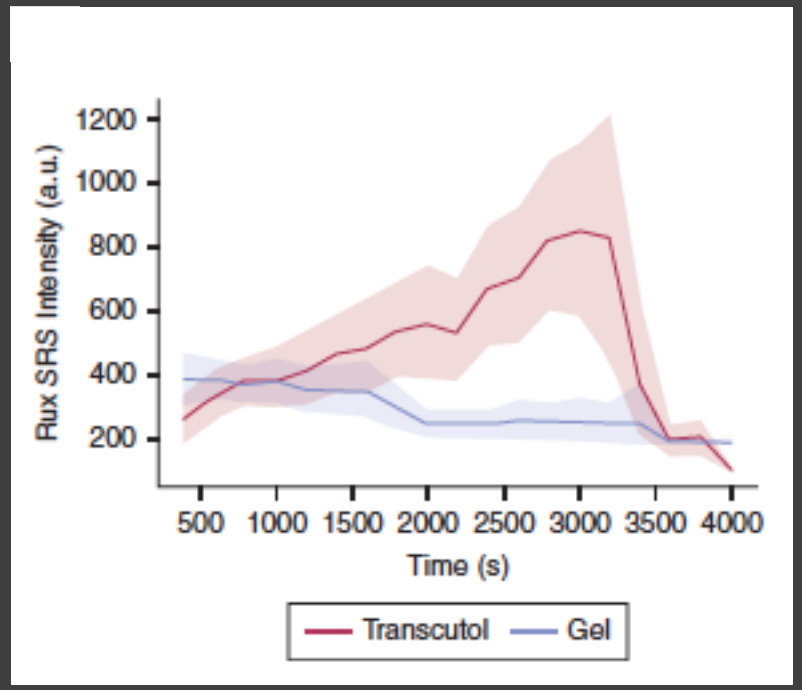


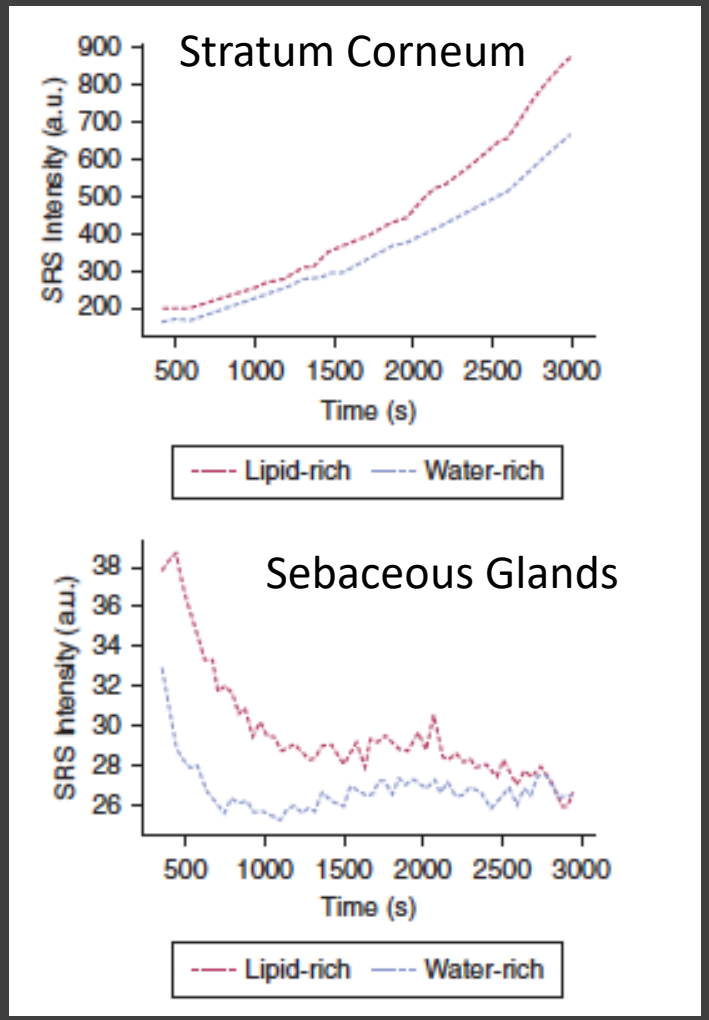
Observe the formation of “particles” at the lipid junctions between corneocytes (Drug in EtOH, drug in delivery gel)

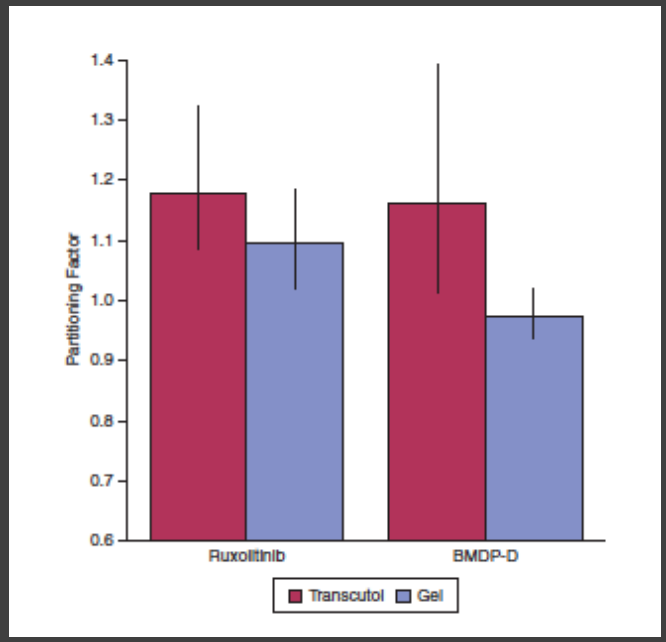
Observing deposition/metamorphosis occurring at the hydrophobic lipid interface

Feizpour, A, et al. *Journal of Investigative Dermatology* (2021)

CRI/Image segmentation allows for cellular level quantification



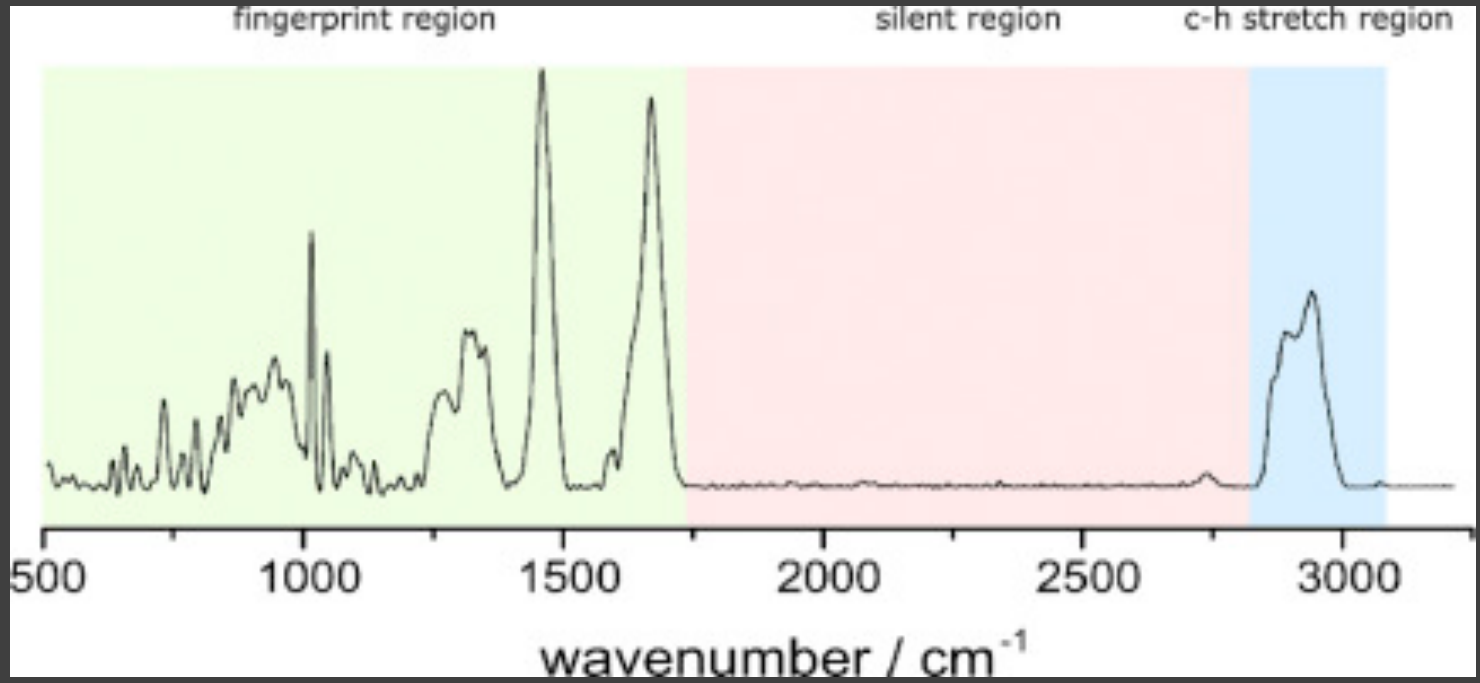




Feizpour, A, et al. *Journal of Investigative Dermatology* (2021)

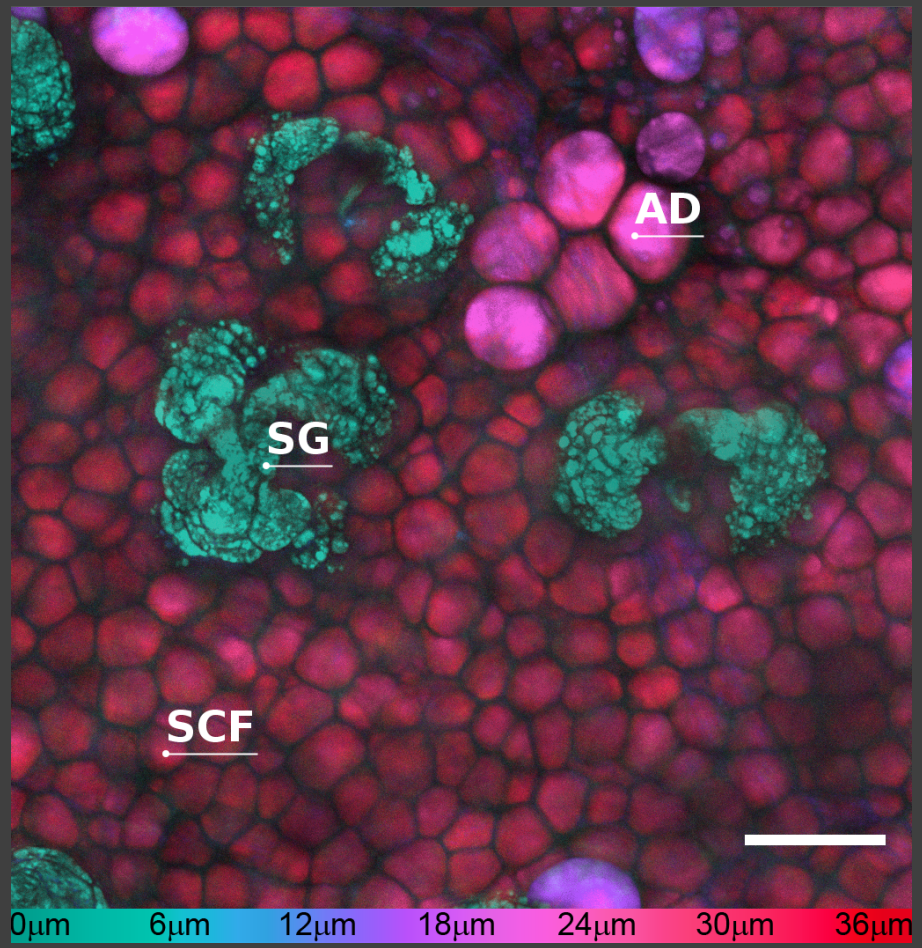
What about those compounds not in silent region?

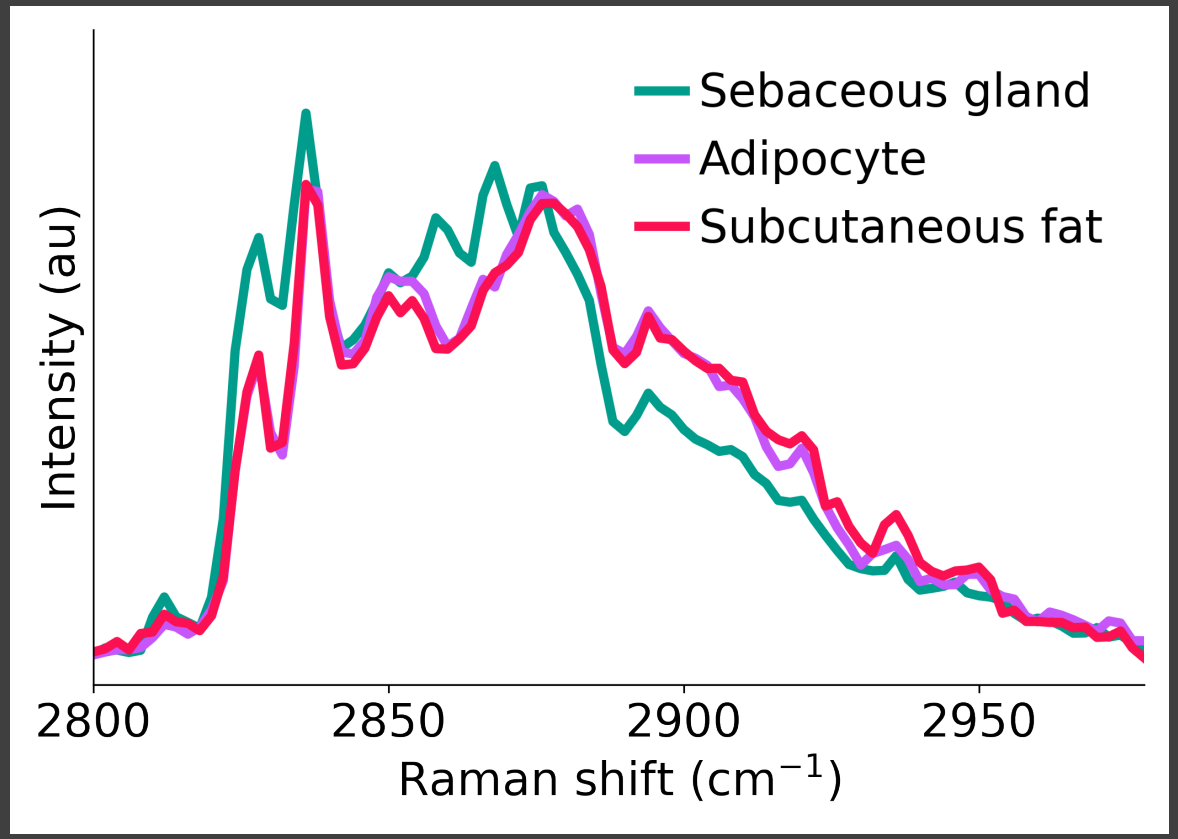
- Can we develop a method to acquire Raman signatures in the fingerprint region and spectrally deconvolve to obtain contributions of each compound?
- Can we track actives and inactive ingredients as they penetration the stratum corneum and permeate deeper into the skin?



Lee, Martin, and William J. Tipping. "Stimulated Raman Scattering for Cell and Tissue Imaging." *Neurophotonics and Biomedical Spectroscopy*. Elsevier, 2019. 401-425.

Hyperspectral SRS to monitor lipid signatures

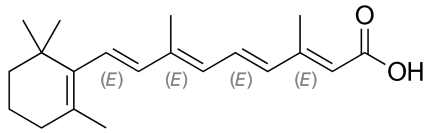




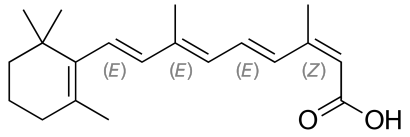
Pence, Isaac J., et al. "Multi-window sparse spectral sampling stimulated Raman scattering microscopy." *Biomedical Optics Express* 12.10 (2021): 6095-6114.

Distinguishing between different APIs

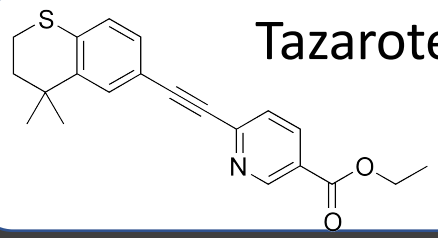
Tretinoin

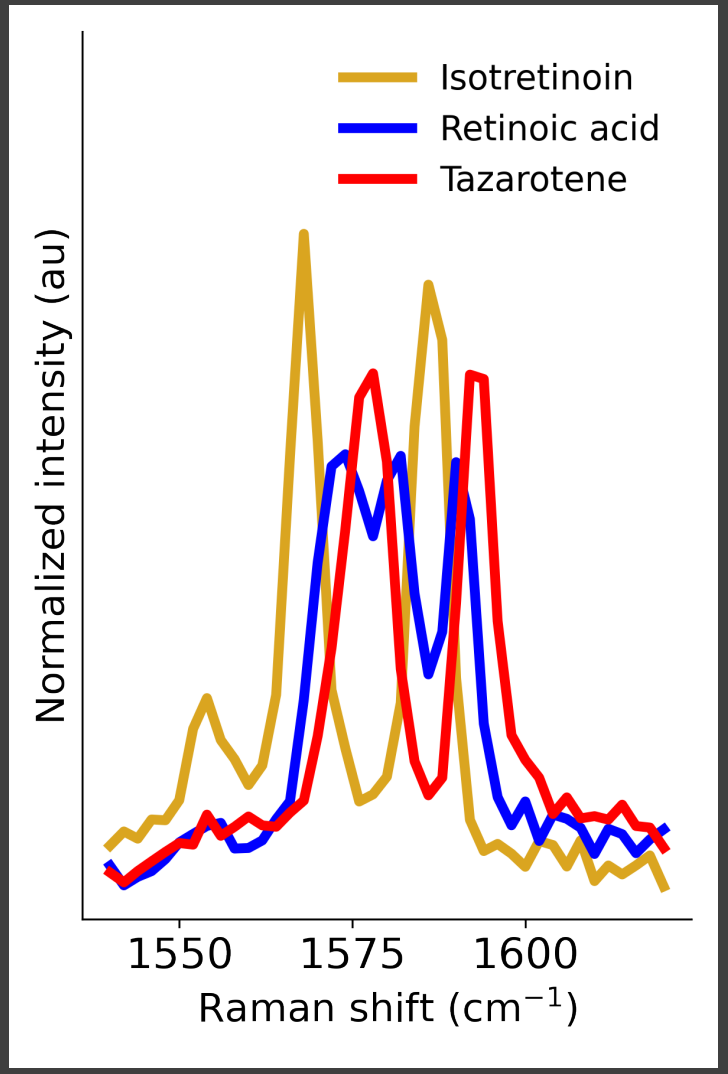


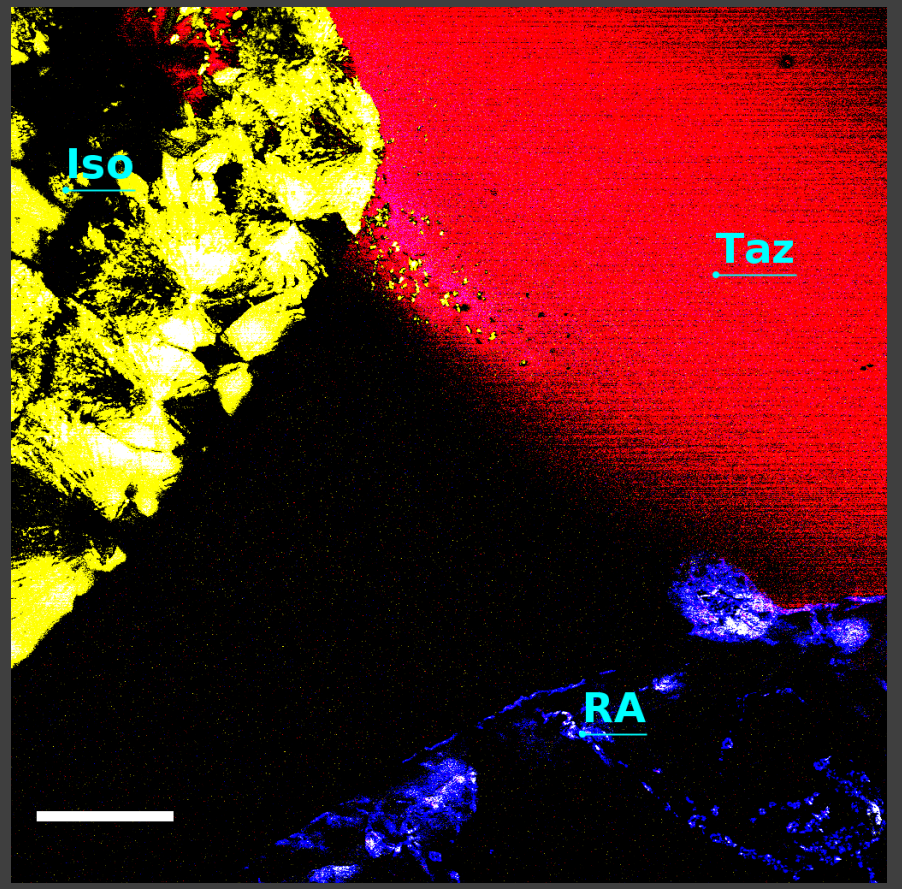
Isotretinoin



Tazarotene







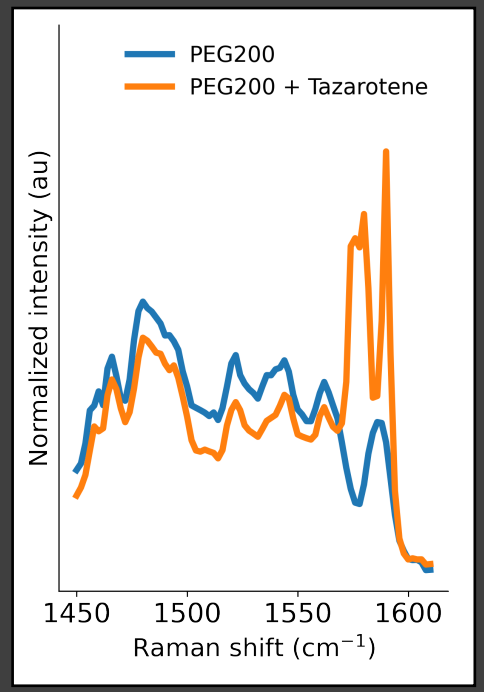
Pence, Isaac J., et al. "Multi-window sparse spectral sampling stimulated Raman scattering microscopy." *Biomedical Optics Express* 12.10 (2021): 6095-6114.

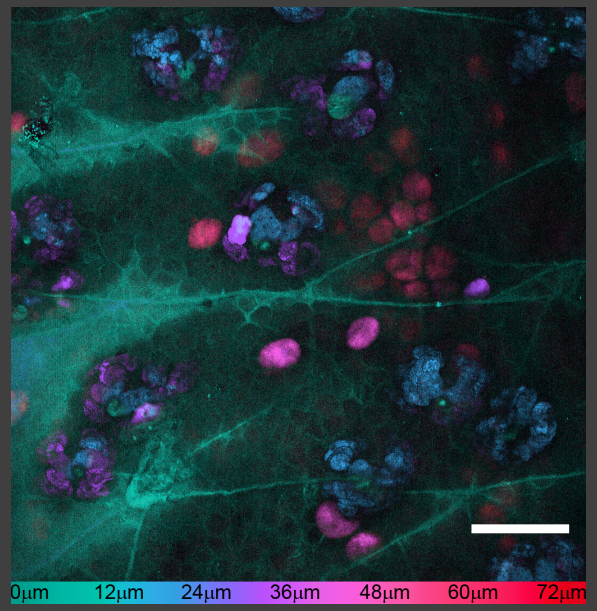
Multicomponent PK Imaging with S4RS

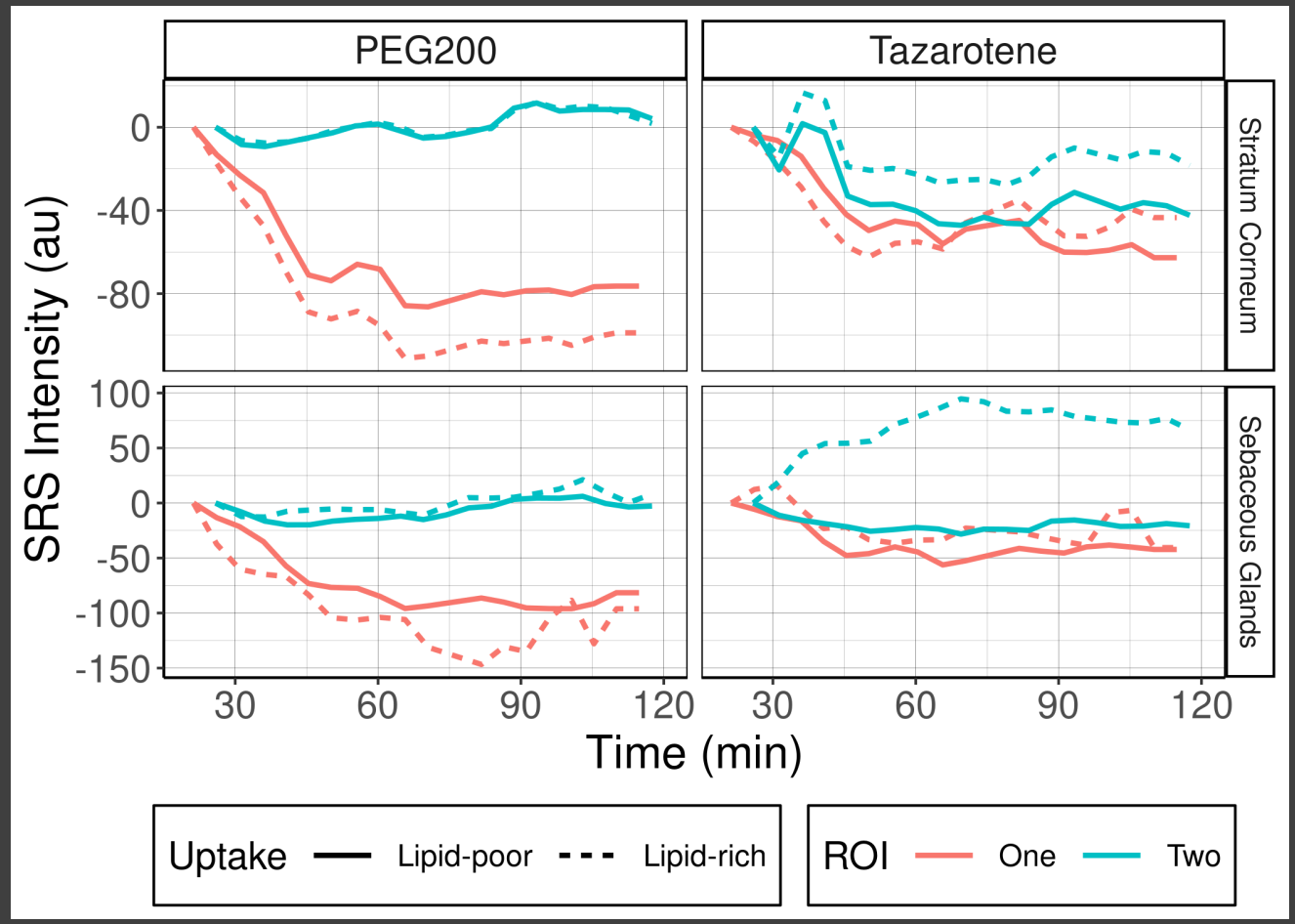
Dynamically acquire S4RS data at specific Raman shifts

Allows selective imaging of:

Lipids (2845 cm^{-1}), tazarotene (1592 cm^{-1}), PEG200 (1482 cm^{-1})







Pence, Isaac J., et al. "Multi-window sparse spectral sampling stimulated Raman scattering microscopy." *Biomedical Optics Express* 12.10 (2021): 6095-6114.

Conclusions

Dermal Microdialysis

- dMD methodology developed here was **sensitive, selective, stable, and reproducible**; however, ruggedness still requires investigation
- Study duration, dose-duration, topical dose, and application site location should be chosen with the utmost care
- Knowledge of the disposition function confirmed a **flip-flop PK scenario** after TDDP application and potentially allows for IVIVRs

Coherent Raman Scattering Imaging

- Compounds that have molecular signatures in the ”silent region” are ideal substrates for CRI
  - Most compounds do not have these signatures and deuteration likely alters the pharmacokinetics
- S4RS methodology has opened numerous possibilities with signal isolation the rate limiting step that can be computationally overcome

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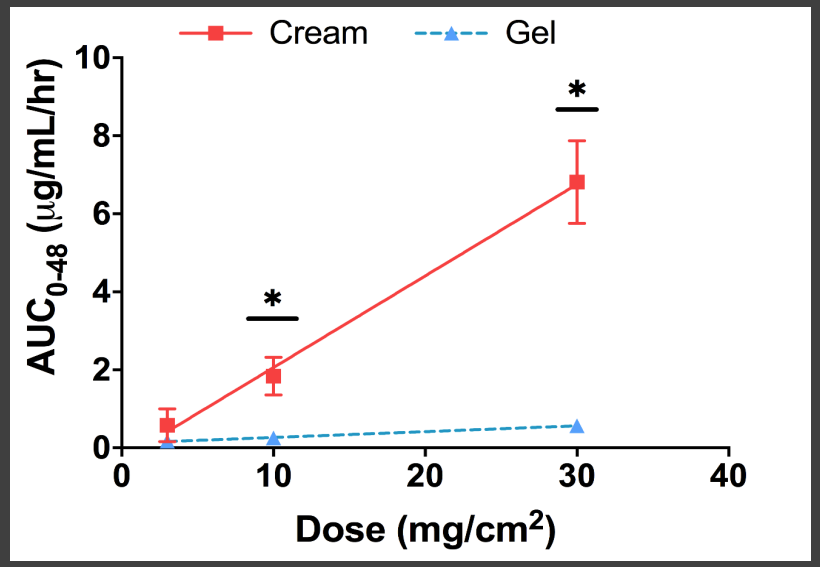
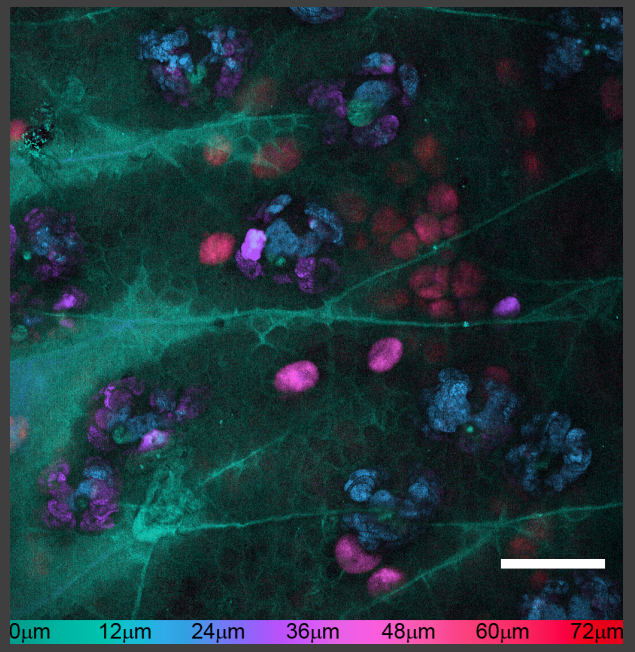
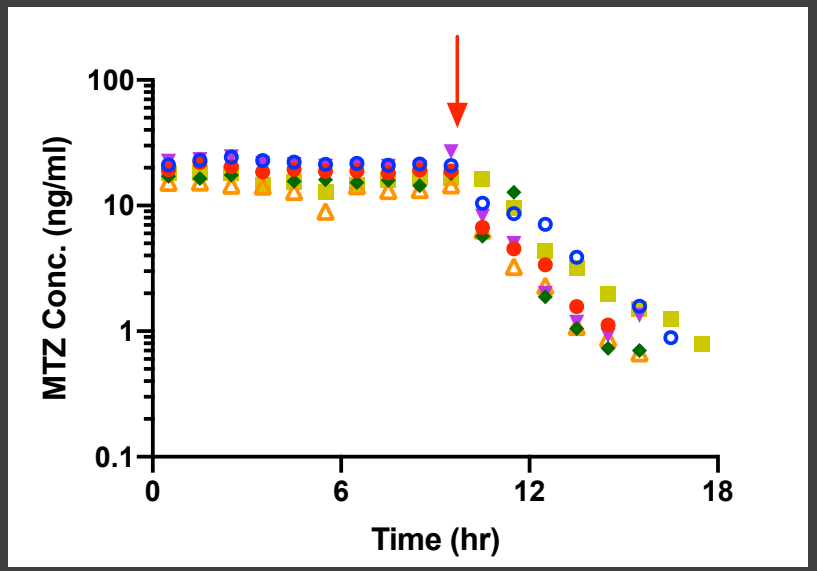
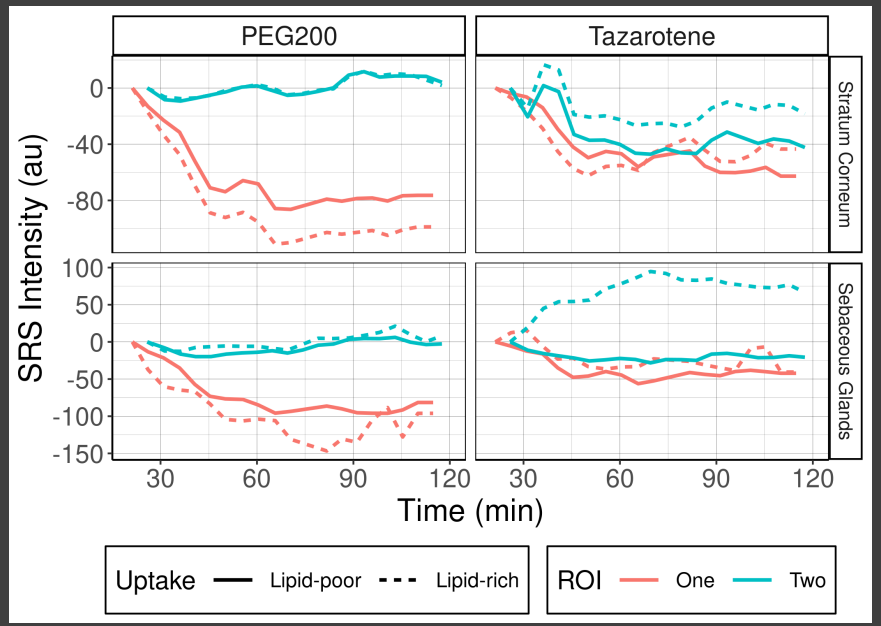
Dr. Kim Troensegaard Nielsen

Questions?


Cream
Gel
AUC_{0-48} (μg/mL/hr)
Dose (mg/cm²)
*
*

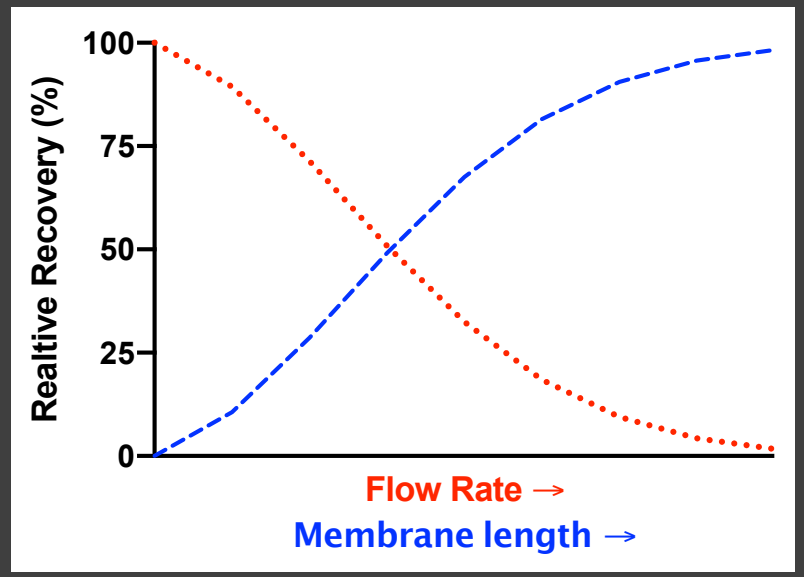

0μm
12μm
24μm
36μm
48μm
60μm
72μm


MTZ Conc. (ng/ml)
Time (hr)

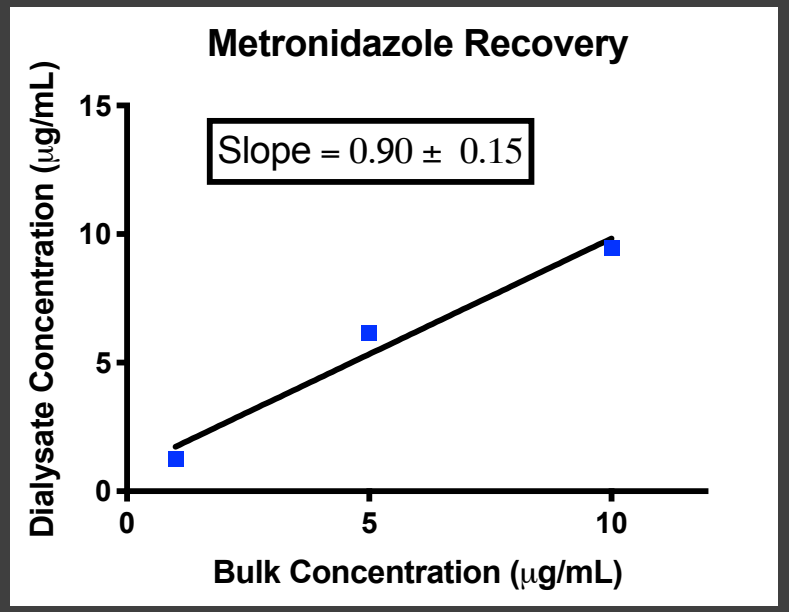

PEG200
Tazarotene
Stratum Corneum
Sebaceous Glands
SRS Intensity (au)
Time (min)
Uptake
Lipid-poor
Lipid-rich
ROI
One
Two

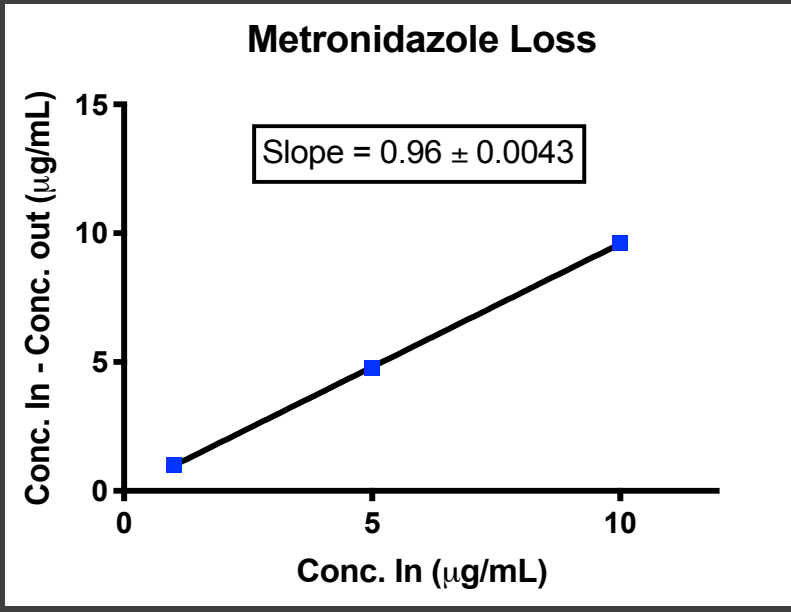
Extras

dMD in vitro system suitability



Optimized Parameters
Flow rate: 0.5 uL/min
Membrane length: 1.7 cm



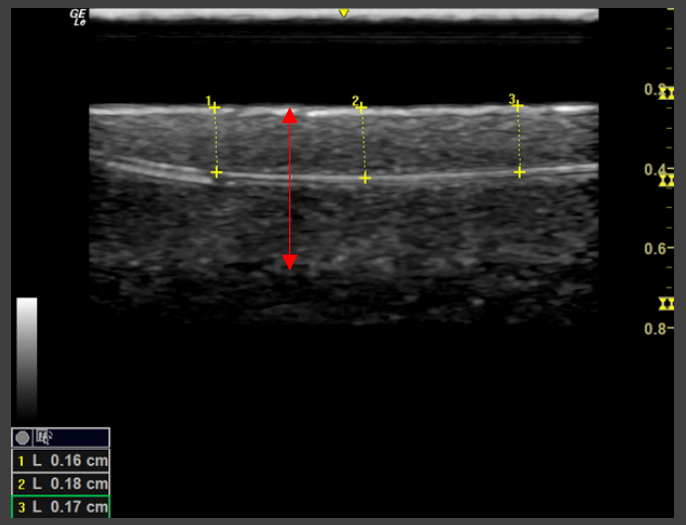


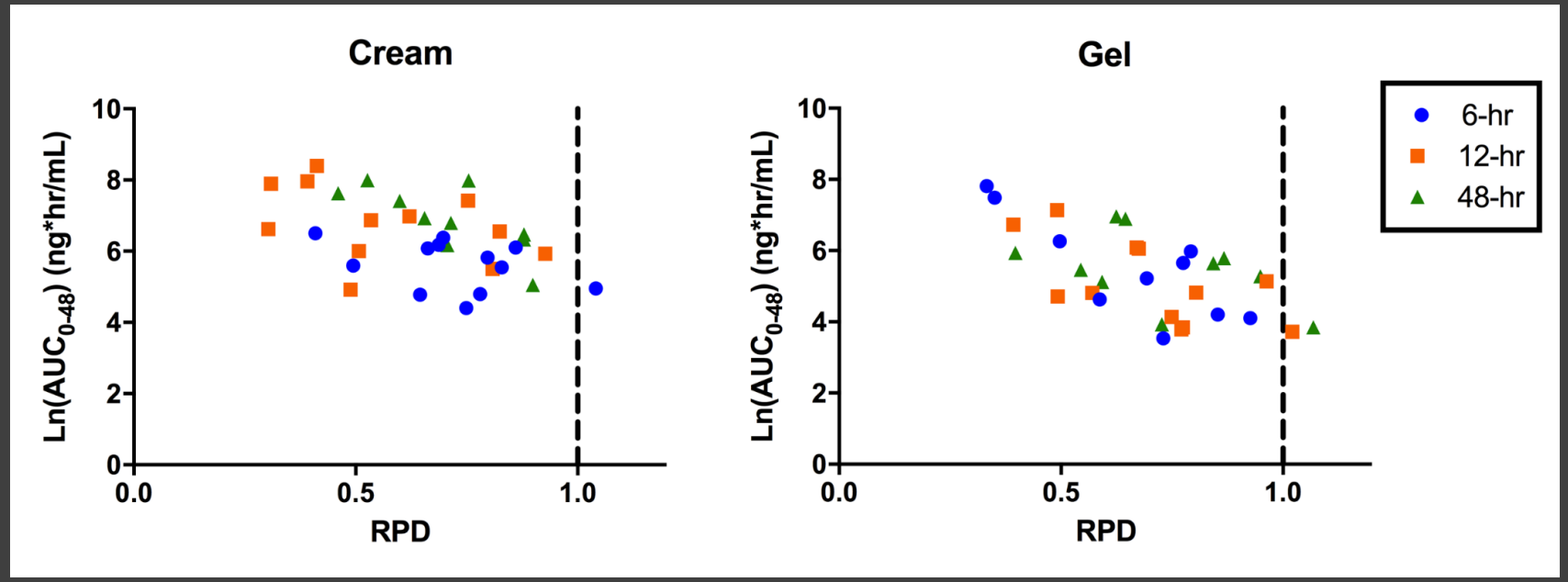
In vitro recovery ~ 90%
In vitro retrodialysis ~ 96%

→ ✓ dMD set-up suitable for Metronidazole

Probe Depth Relationship

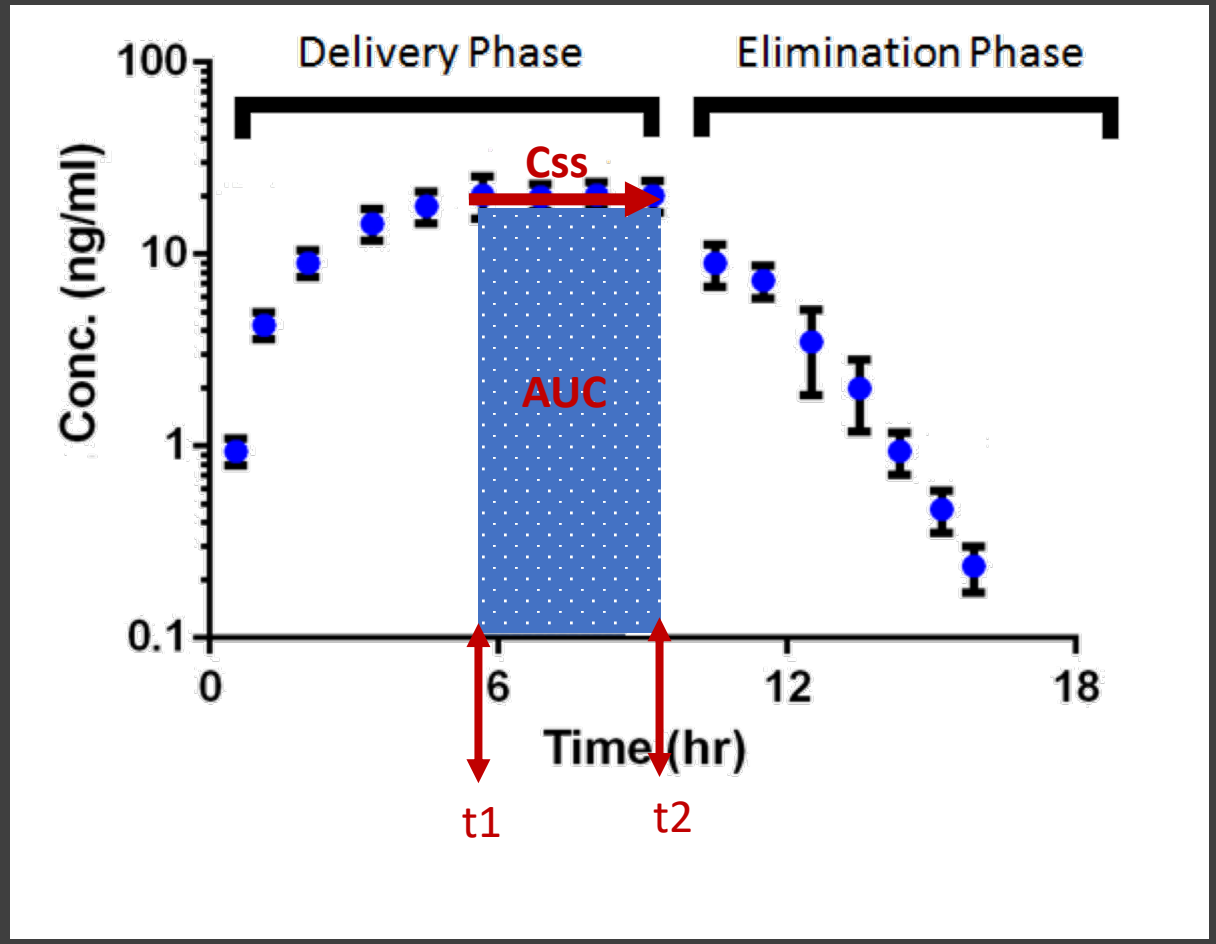
- From ultrasound images we can measure both probe depth and skin thickness and arrived at a relative probe depth (RPD)
- Only moderate correlation ($R^2 = 0.6$) for the 6-hr gel but no real correlation for other dosing schemes ($R^2 < 0.5$) comparing $Ln(AUC_{0-48})$ vs RPD





Dermal API delivery via dMD probe

- Identify steady state concentration (C_{ss})
- Measure the AUC under the selected steady state (shaded area)
- Calculate the dose delivered in that time interval:
  - $Dose_{t1-t2} = (C_{perfusate} - C_{ss}) \times V_{perfused\ (t1-t2)}$
- Calculate clearance:
  - $Cl = \frac{Dose_{t1-t2}}{AUC_{t1-t2}}$
- Fit the best poly-exponential equation to the elimination-phase data;
- Estimate V_d:
  - E.g., if mono-exponential: $V_d = \frac{Cl}{k_e}$
  - More complicated if poly-exponential



dUIR Calculation

- UIR for mono-exponential elimination:

$$UIR = \frac{1}{V_d} \times e^{-k_e t}$$

Where V_d has units of mL and K_e has units of hr^{-1}

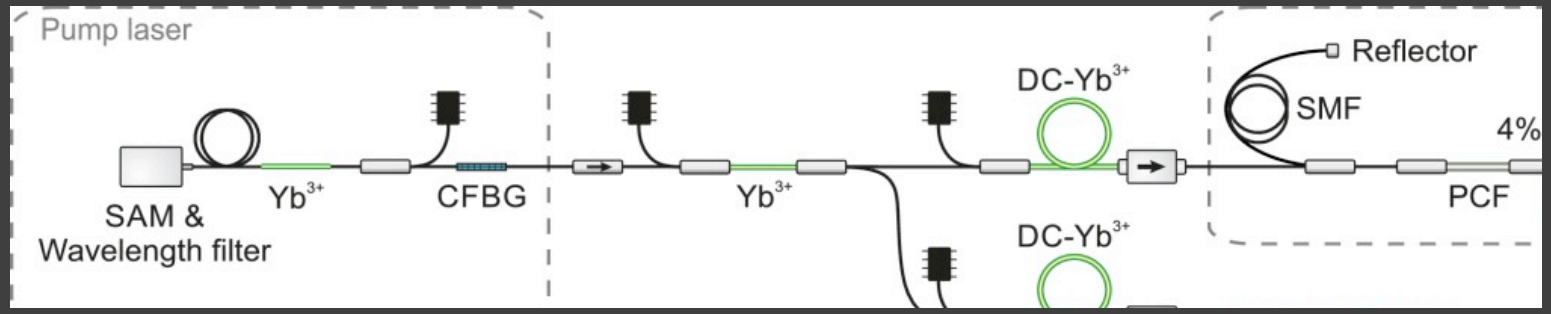
- Averaged dUIR for all probes and subjects:

$$dUIR = 10.1 \times e^{-0.47t}$$

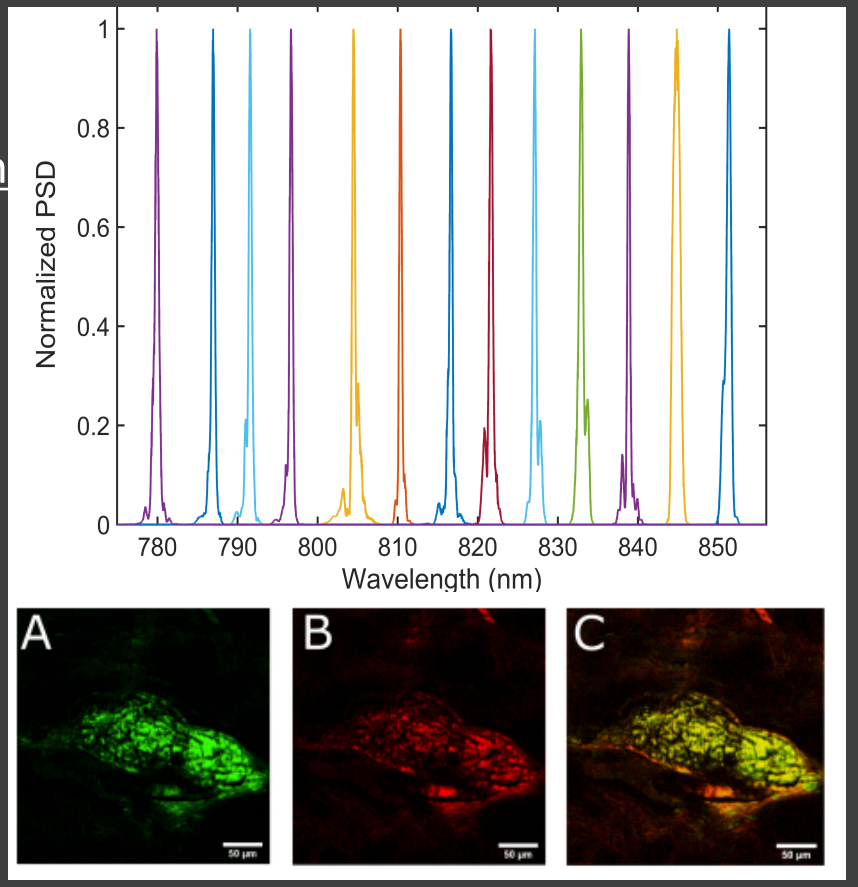
- dUIR can be used to deconvolve **dermal microdialysis concentration data**
- dUIR can also be used to convolve **in vitro permeation testing data**

New Fiber Laser for Imaging

- All fiber-OPO offers stable dual output and rapid (<5ms) wavelength tun changing the pump laser repetition rate – *electronic adjustment*
- *5 ms per wavelength jump*
- >100 mW output powers for both pump and Stokes beams
- 19” RU enclosure for direct mounting & compactness



Maximilian Brinkmann, *et al.*, "Portable all-fiber dual-output widely tunable light source for coherent Raman imaging," Biomed. Opt. Express 10, 4437-4449 (2019)



Images of human sebaceous gland from 30 m thin skin tissue sections. (a) CARS image obtained by tuning to 2845 cm^{-1} (symmetric CH_2). (b) CARS image obtained by tuning to 2934 cm^{-1} (asymmetric CH_3). (c) Merged two-color image from (a) and (b) revealing heterogeneous distributions of lipids (green/yellow) and proteins (orange/red).