

# **Biological Sample Collection**

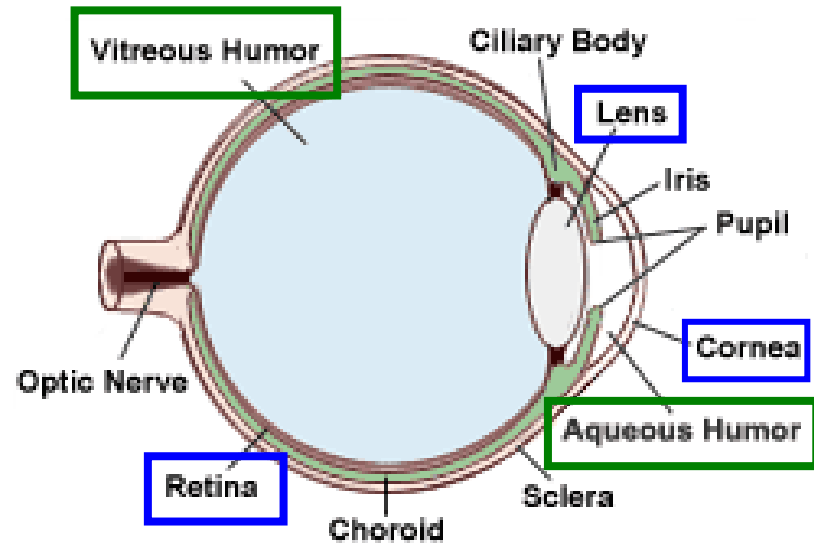
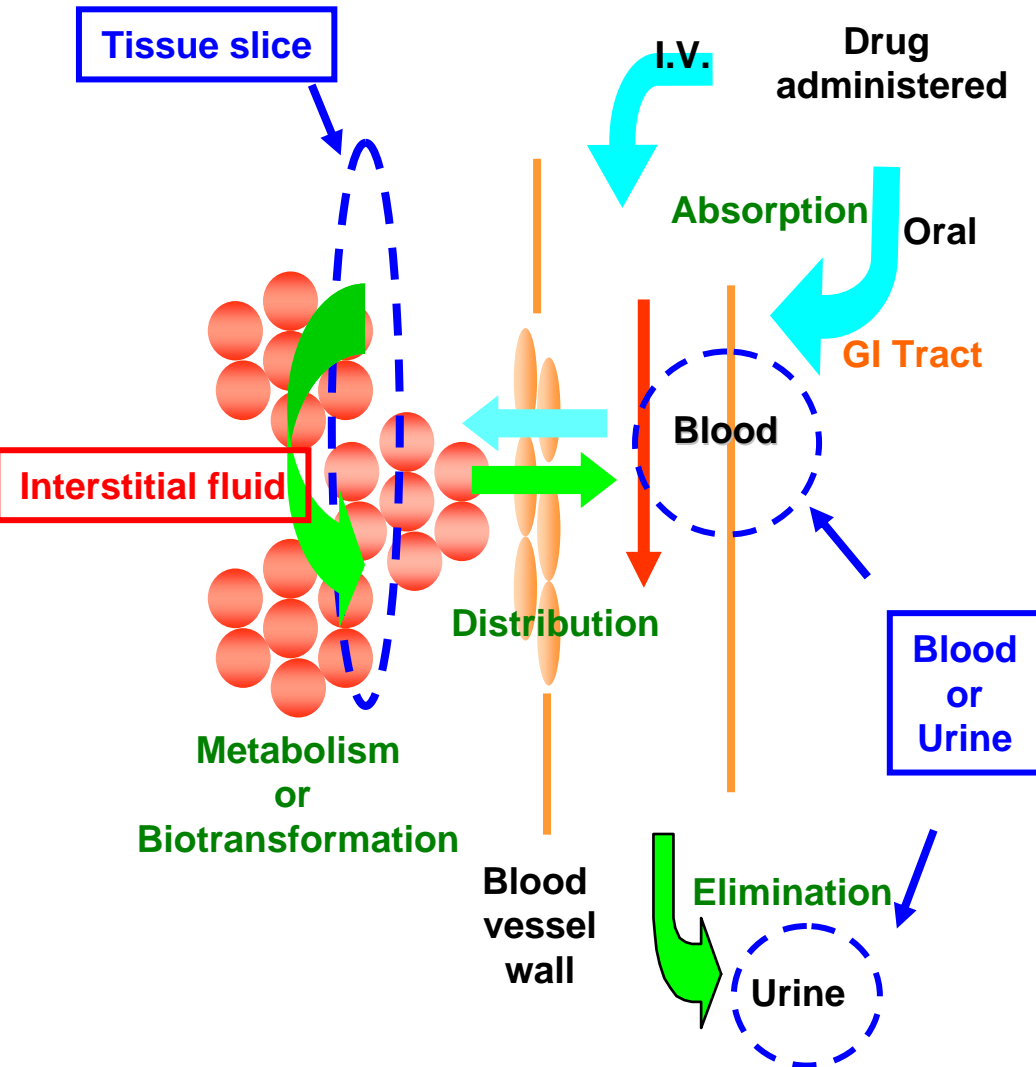
## **~ Interstitial Fluids & Aqueous Humor ~**

**Chao-Cheng (Sam) Wang, PhD**

**Department of Dermatology  
UAB**

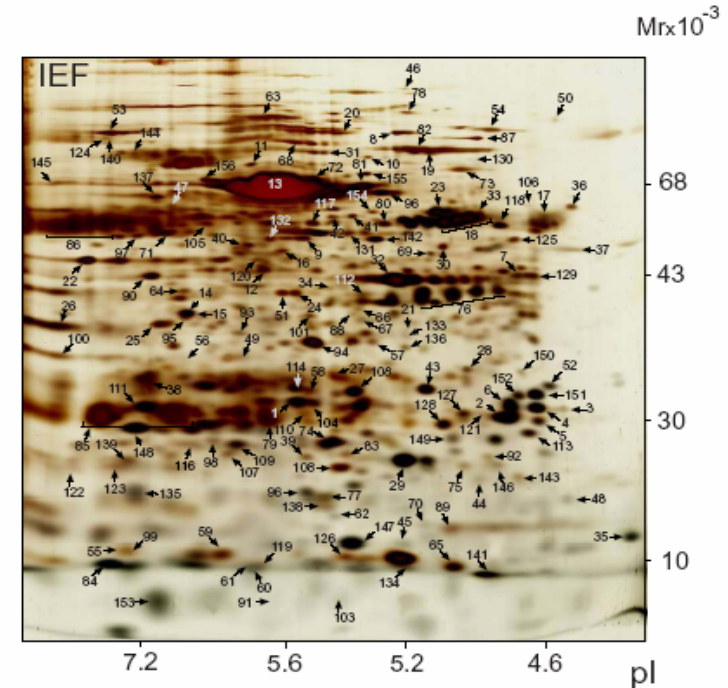
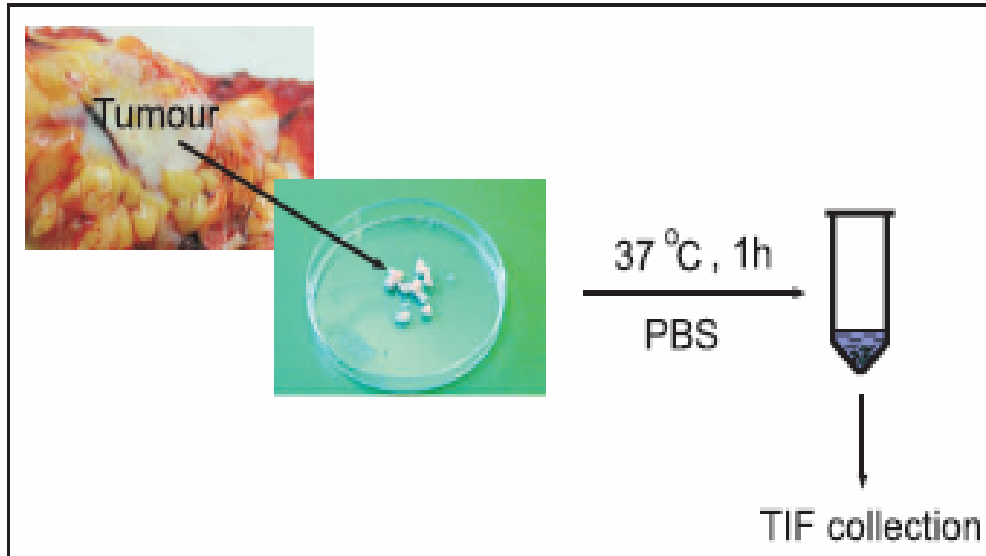
**Purdue-UAB Botanical Workshop, September 11-12, 2006**

# Targets for Biological Sampling



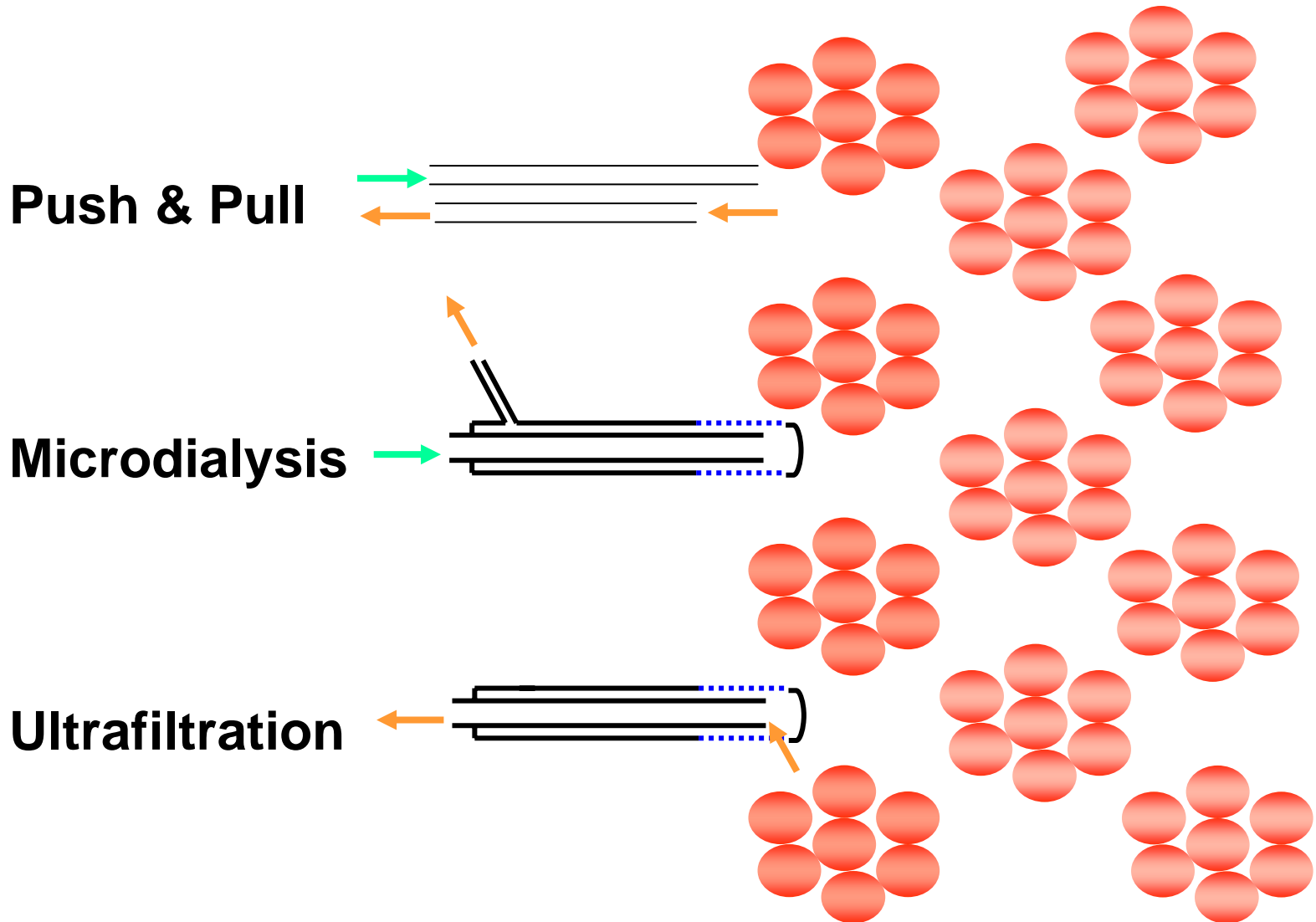
# Fluids in Interstitial Spaces

## Ex-vivo Interstitial fluid collection:



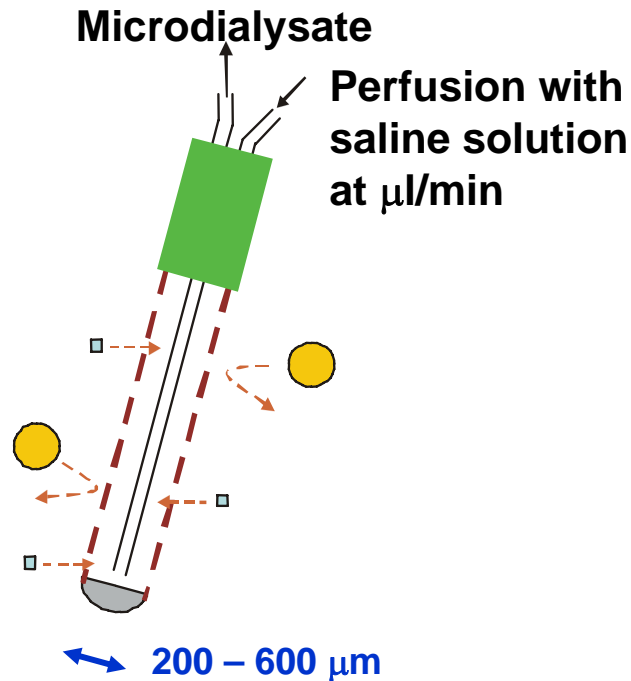
- Complexity of proteins in interstitial space.
- ex-vivo sampling technique.
- Difficulty in quantitative analysis
- Difficulty in obtaining multiple samples from the same tissue at different disease stages.

# *In-vivo* Interstitial Fluid Sampling

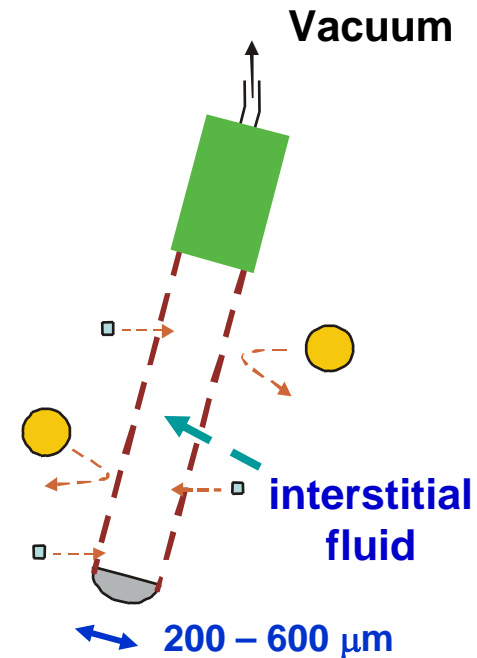


# In-vivo Sampling from Interstitial Space

## Microdialysis (MD)



## Ultrafiltration (UF)

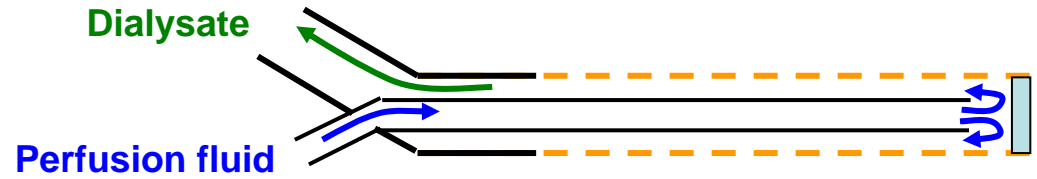


### Advantage:

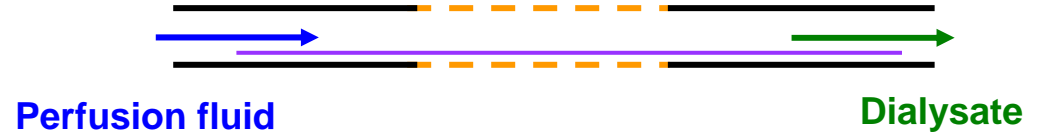
- Sampling free drug or metabolites (non-protein-bound) in interstitial fluid at the site of interest.
- Excellent temporal resolution for PK studies from single animal.
- Real *in-vivo* sampling from live, freely-moving animals.

# Microdialysis probes

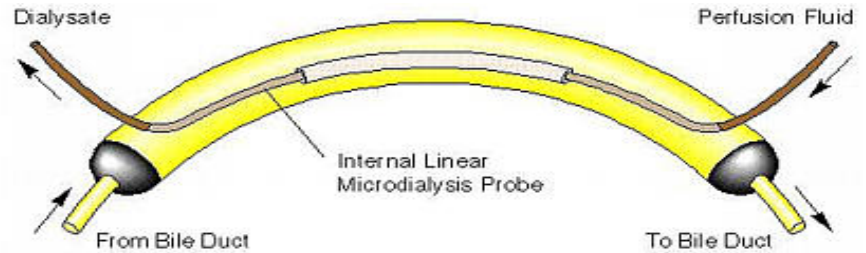
## Concentric probe



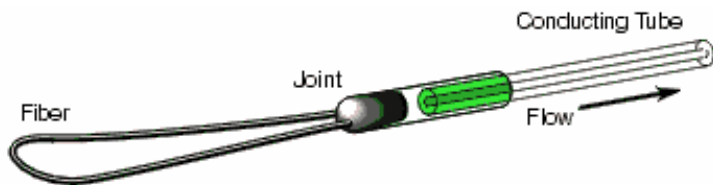
## Linear probe



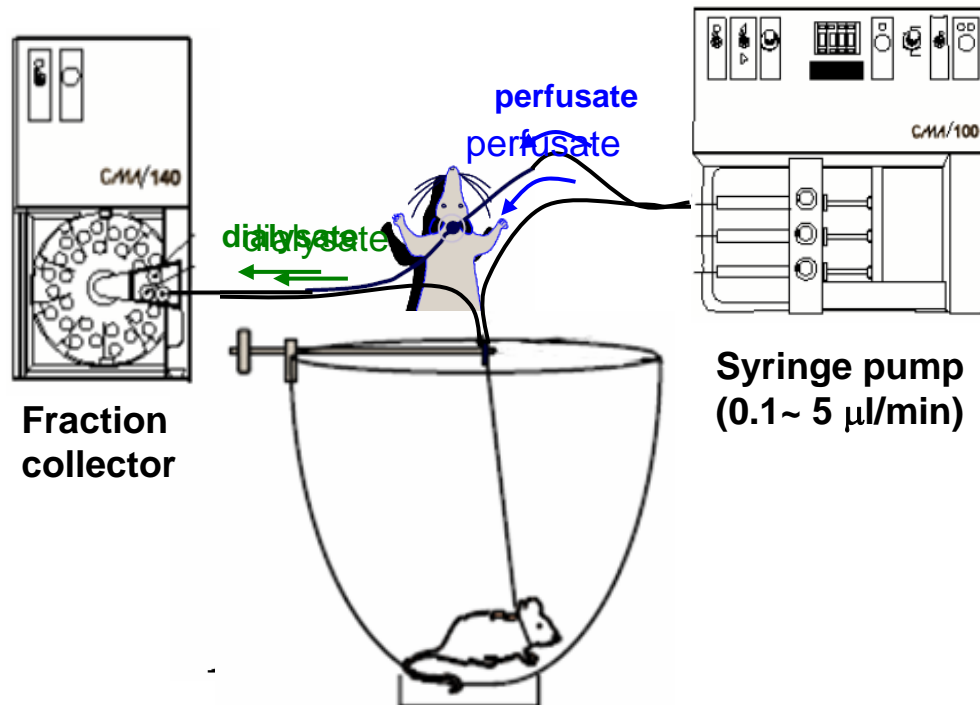
## Shunt probe



# Ultrafiltration probes

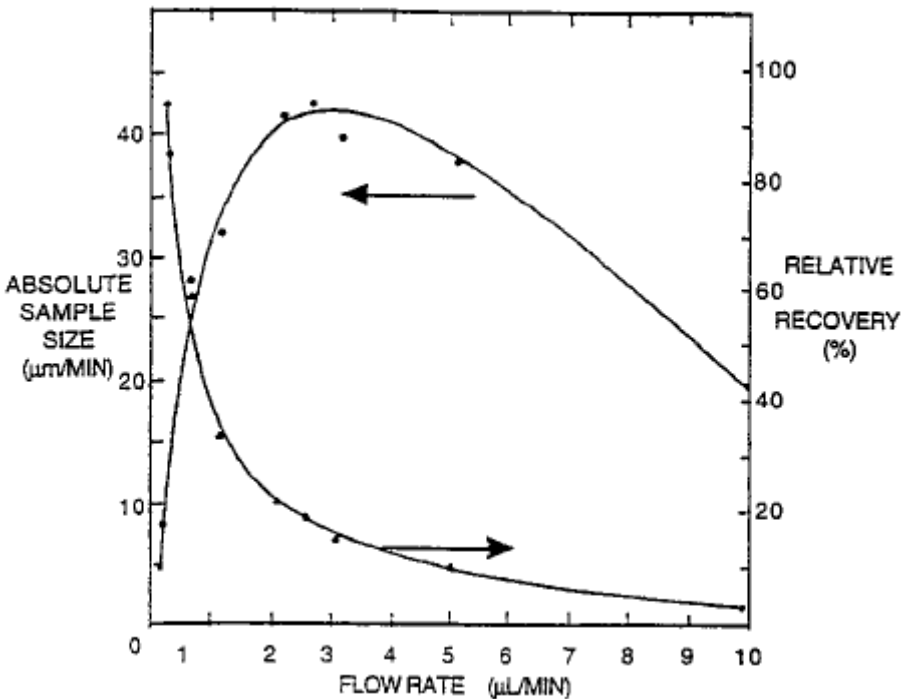


# Experimental Setup for *In-vivo* Sampling from Interstitial spaces



# Recovery Characterization

~ relative recovery (%) & absolute recovery ( $\mu\text{mol}/\text{min}$ ) ~



## Calibration methods:

- Flow rate method
  - Constant  $C_{\text{sample}}$
  - Various Flow rate
- Zero-net flux method
  - Constant flowrate,  $C_{\text{sample}}$
  - Various  $C_{\text{perfusate}}$
- Retrodialysis method
  - standard compound in perfusate
  - $$\text{Recovery} = 1 - \frac{(C_{\text{perfusate}} - C_{\text{dialysate}})}{C_{\text{perfusate}}}$$



# Microdialysis vs Ultrafiltration

## Advantage:

- Sampling free drug or metabolites (non-protein-bound) in interstitial fluid at the site of interest.
- Excellent temporal resolution for PK studies from single animal. (reduced # of animal usage)
- Real *in-vivo* sampling from live, freely-moving animals.

## Microdialysis:

diffusion-based technique

- poor recovery
- not suitable for long term *in-vivo* sampling.

## Capillary Ultrafiltration:

non-diffusion-based technique

- better & consistent recovery
- suitable for long term sampling (up to 6 month).

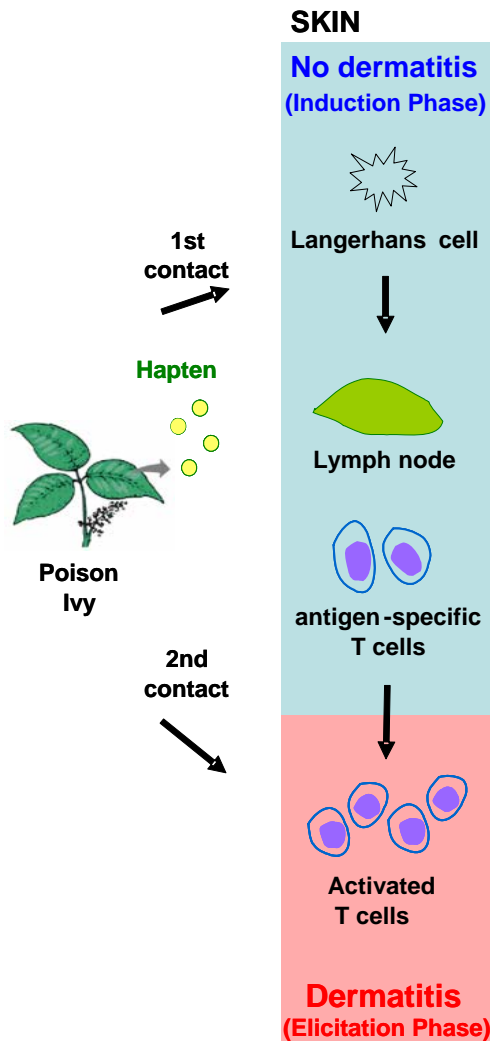
# Applications

- **Small molecular:**  
(MWCO < 20 kda)
  - **Endogenous compounds:**
    - **Neurotransmitter:**  
dopamine.....
    - **Biological markers:**  
lactate...
  - **Exogenous compounds:**
    - **Therapeutic drugs**
- **Large molecules:**  
(MWCO > 400 kda)
  - **Cytokines**
  - **Proteins**
- Brain (animal models and human)
- Muscle (animal models)
- Heart
- Lung
- Kidney
- Liver
- Blood
- Skin (animal models and human)
- Eye
- Other peripheral tissues

# Bibliography

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- Bioanalytical System ([www.bioanalytical.com](http://www.bioanalytical.com))

# Dynamic CUF Sampling from ACD Model



**Animal: C3H/HeN mouse**

**Hapten: DNFB** (dinitrofluor-benzene)

**control/control, control/DNFB, DNFB/DNFB**

**Day 1 & 2**

**Induction:**

0.5 % DNFB (in 20 % olive oil in acetone)

- 25  $\mu$ l on shaved abdominal skin and footpads

**Day 6 (the day before elicitation)**

**UF probe implantation:**

CUF probe was implanted subcutaneously in ear and housed in a freely-moving system with access to water and food

- collect interstitial fluid (IF) samples 12 hours before elicitation

**Day 7**

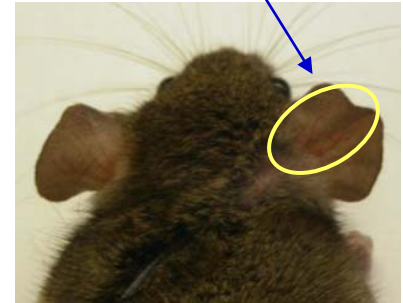
**Elicitation:**

0.2 % DNFB (in 20 % olive oil in acetone)

- 10  $\mu$ l on both side of each ear

- IF samples were collected continuously into 24 hours intervals for 3 days

**CUF probe implanted**



1. Ear thickness were measured daily before and after DNFB elicitation.
2. UF samples were processed with 2DE cleanup kit and analyzed with 75  $\mu$ g protein load on 3-10 IEF/ 12.5% SDS gel / Sypro staining.

# 2DE Analysis of IFs from different ACD Stages

