Tissue and Fluid Proteomics

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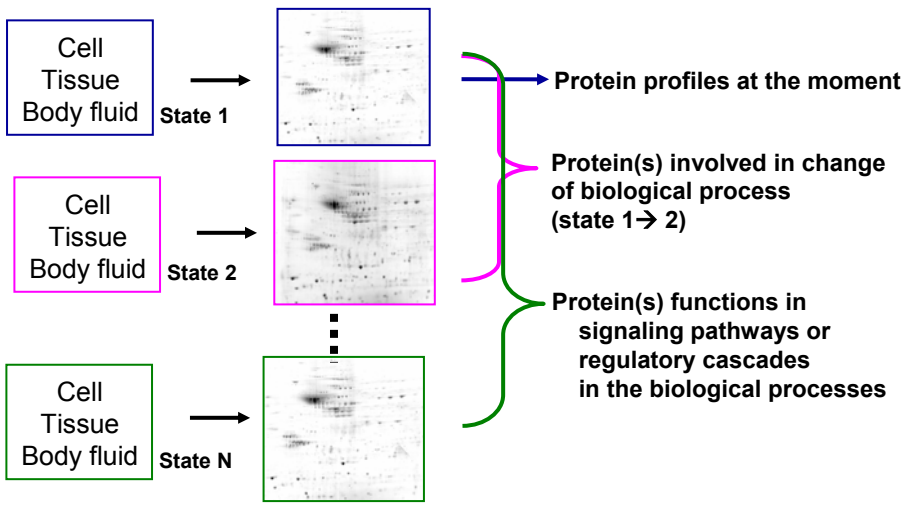
Proteomics: Picture (s) to Movie



These unique changes of proteins can serve as

- **markers for early detection and predictive purposes**
- **novel targets for drug discovery and therapeutic intervention**

Sample Sources for Proteomic Analysis

- Cell lines.
- Tissue sections.
- Body Fluids:
  - Blood and urine.
  - Fluids from secretion.
  - Fluids in interstitial spaces.

Fluids from Secretion

- Aqueous Humor
  - AH was collected by 27 G needle (150 μl) from patients w/ or w/o corneal rejection.
  - 2D gel w/ MS ID.
  - Funding et al., Acta Ophthalmol. Scand. (2005) 83, 31-39.
- Saliva
  - Whole saliva or major salivary gland secretions.
  - 2D gel w/o or w/ MS ID; LC-MS^n.
  - Proteome database for biomarkers of specific diseases.

Fluids from Secretion

- Cerebrospinal fluids (CSF)
  - Fluid surrounding the central nervous system.
  - Total vol ~140 ml, produced at 03-0.4 ml/min.
  - Samples were collected by lumbar puncture (10-12 ml).
  - 2D gel w/ MS ID; LC-MS^n
  - Studies of the path-physiological mechanism in front-temporal dementia, Alzheimer's disease
  - Yuan et al. J Chromatogr B , (2005) 815(1-2),179-89. (review)

- Synovial fluid
  - A dynamic reservoir for proteins originating from serum, synovial tissue, and cartilage.
  - 2D gel / MS ID and LC^n-MS^n.
  - Study for biomarkers for Rheumatoid Arthritis.
  - Tilleman et al. Rheumatology (Oxford), (2005) 44,1217-26. (review) .

Fluids from Secretion

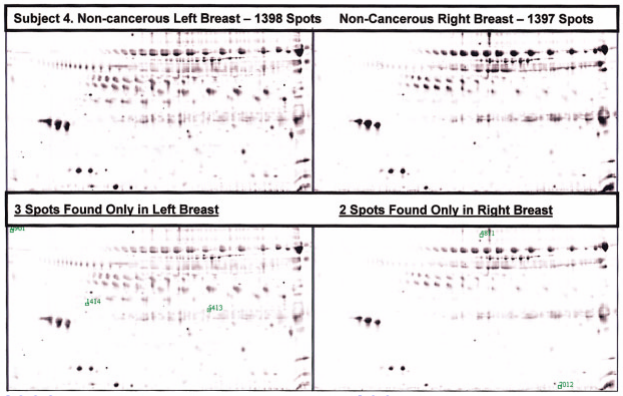
- Bronchoalveolar lavage (BAL) fluids
  - Obtained by washing the epithelial lining of lung with PBS.
  - 2D gel / MS ID and LC^n-MS^n.
  - Studies of fibrosing interstitial lung diseases, such as sarcoidosis, and allergic asthma.
  - Wattiez et al. J Chromatogr B (2005) 815, 169-178. (review)

- Nipple aspiration fluid (NAF)/ Ductal lavage fluid
  - NAF: breast ductal fluid collected by nipple aspiration.
  - Non-invasive way of sample collection.
  - NAF: sample vol: generally ~ 10-20 μl.
  - 2D gel, SELDI, and chromatography-MSMS.
  - Studies of the early diagnosis of breast cancer.

Review: Human body fluid proteome analysis. Proteomics. 2006 Dec;6(23):6326-53

Protein Profiles of Bilateral Matched Paired NAFs by 2DE-Approach

Non-Cancerous Left Breast **Non-Cancerous Right Breast**



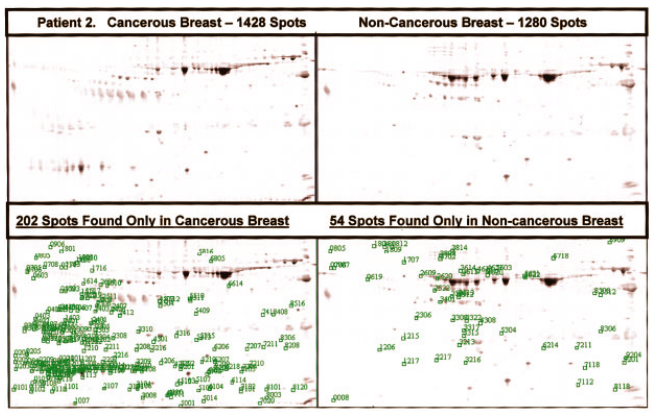
1398 spots
3 spots found only in Left Breast

1397 spots
2 spots found only in Right Breast

Kuerer, HM; et al., *Cancer* (2002) 95, 2276-2282.

Protein Profiles of Bilateral Matched Paired NAFs by 2DE-Approach

Cancerous Breast **Non-Cancerous Breast**



1428 spots
202 spots found only in Cancerous Breast

1280 spots
54 spots found only in non-Cancerous Breast

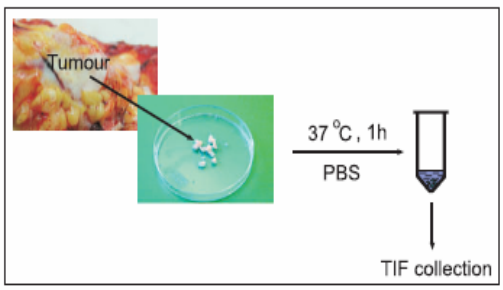
Kuerer et al., *Cancer* (2002) 95, 2276-2282.

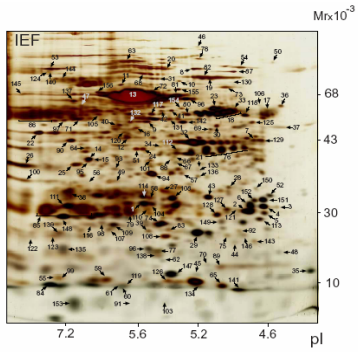
Approaches for Sampling from Extra-cellular Space (Interstitial Fluids)

- **Tissue perfusion (TIF)**
- ***In-vivo* sampling from interstitial space with capillary probes (CUF)**

Fluids in Interstitial Spaces

Ex-vivo Interstitial fluid collection:



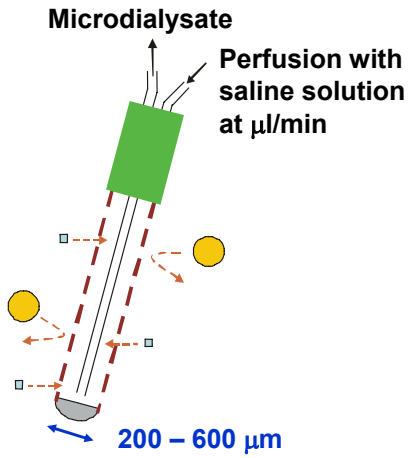


- **Complexity of proteins in interstitial space.**
- **ex-vivo sampling technique.**
- **Difficulty in obtained samples from the same tissue at different disease stages.**

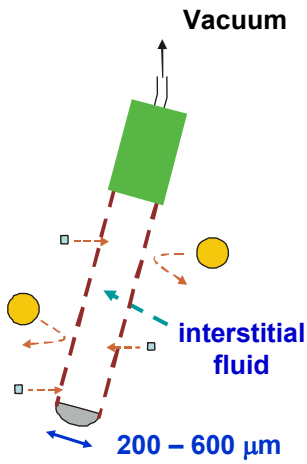
Celis et al., Mol Cell Proteomics. 2004, 327-344.

In-vivo Sampling from Interstitial Space

Microdialysis (MD)



Ultrafiltration (UF)



(small molecules analysis)

Microdialysis vs Ultrafiltration for Proteomic Sampling

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:

diffusion-based technique

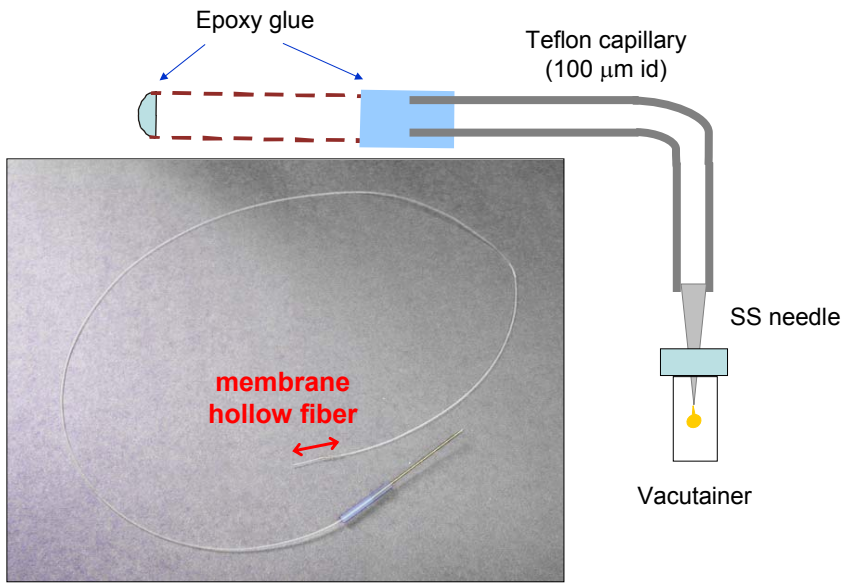
- poor recovery (for peptides and proteins)
- not suitable for long term in-vivo sampling.

Capillary Ultrafiltration:

non-diffusion-based technique

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

Capillary Ultrafiltration Probe



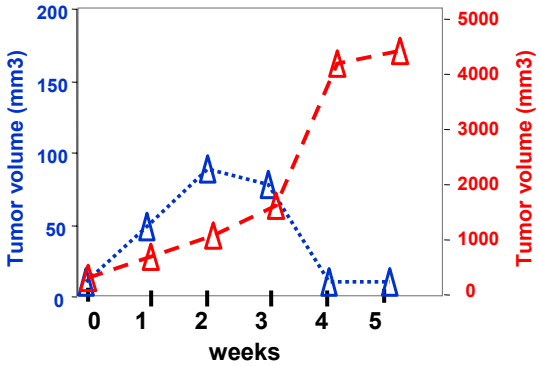
CUF Sampling from Animal Models

- *In-vivo* UF sampling from interstitial microenvironment in tumor masses at different developing stages.
- Continuous UF sampling from a freely-moving mouse model with chemical-induced Allergic Contact Dermatitis.

Regressive Skin Tumor Model (C2240)

C3H/HeN Mice

Nude mice



Dynamic interaction between Tumor and Host cells

UAB Center of Skin Diseases

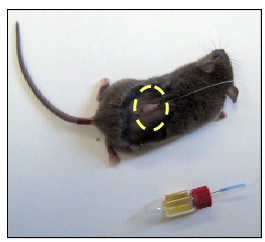
CUF Sampling from Regressive Tumor Model

C3H/HeN Mice

C2240 skin tumor cell

500,000 cells/50µl Inj s.c. on the back

1. Measure tumor size
2. Implant CUF probe in tumor masses for IF collection.



- **Tumors grew on WK 1, but tumor masses decreased after WK2.**
- **Interstitial fluid in tumor was collected by a high MWCO probes for 3 hours.**

~ 2–5 µl of IF collected

tryptic digestion

Nano-LC-qTOF MS for peptide sequencing

Protein ID through PROWL & SWISS-PROT database

Secretomes from Regressive Skin Tumors

Tumors progress at 1st week

1. **S100A4** (Metastasis-associated calcium binding protein)
2. **Thymosin β4**
3. **Thymosin β10**
4. **Profilin 1** (dendritic exosomes)
5. **beta 1-globin**
6. **Hemoglobin β2**

Tumors regress at 3rd week

1. **Fetuin-A** (α-2HS-glycoprotein)
2. **Apolipoprotein A-1**
3. **Alpha-antitrypsin**
4. **Contrapsin** (Trypsin inhibitor)
5. **beta 1-globin**
6. **Hemoglobin β2**

All are secretory proteins

Underline: tumor associated proteins

CUF can be used to sample clean interstitial fluid *in-vivo* from animals at different physiological / disease stages

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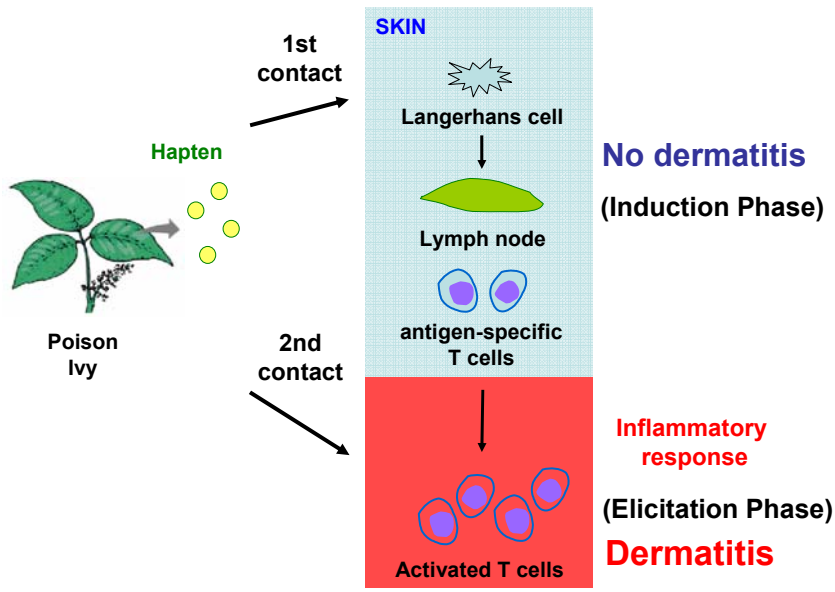
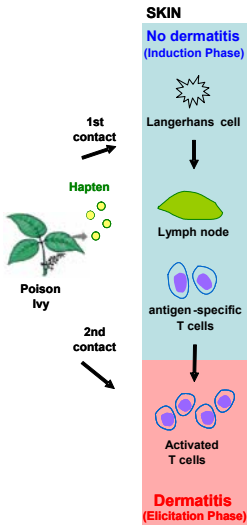
Allergic Contact Dermatitis



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 μl 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 μl on both side of each ear
- IF samples were collected continuously into 24 hours intervals for 3 days

CUF probe implanted

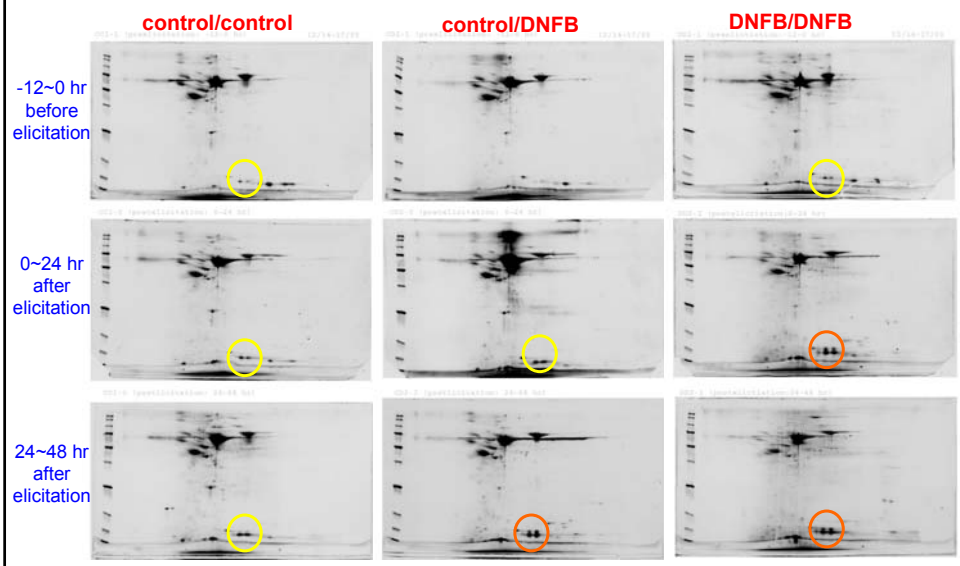
1. Ear thickness were measured daily before and after DNFB elicitation.
2. UF samples were processed with 2DE cleanup kit and analyzed with 75 μl protein load on 3-10 IEF/ 12.5% SDS gel / Sypro staining.

Ear Swelling of ACD Model

	CC-1	CD-1	CD-2	DD-1	DD-2
24 hrs after elicitation	0/1	0/2	0/2	4/-	7/10
48 hrs after elicitation	0/2	0/0	1/2	7/-	5/5

1. Left ear / Right ear (with Probes), unit: 0.01 mm.

2DE Analysis of IFs from different ACD Stages



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Protein ID of IFs from different ACD Stages

Protein	Mass	MS	MOWSE
Cp protein	121074	MALDI	126
Gsn protein	80712	MALDI	114
Plasminogen	90723	MALDI	88
transferrin	76628	MALDI	140
albumin 1	68678	MALDI	167
vitamin D-binding protein	53051	MALDI	82
kininogen 1	47868	MALDI	74
Serpina1a protein	45593	MALDI	103
apolipoprotein A-IV	44545	MALDI	168
gamma-actin	40992	MALDI	135
apolipoprotein A-I	30569	MALDI	118
trophoblast specific protein beta	13802	MALDI	66
vitamin D-binding protein	53085	Q-TOF	
Calgranulin B	12909	Q-TOF	

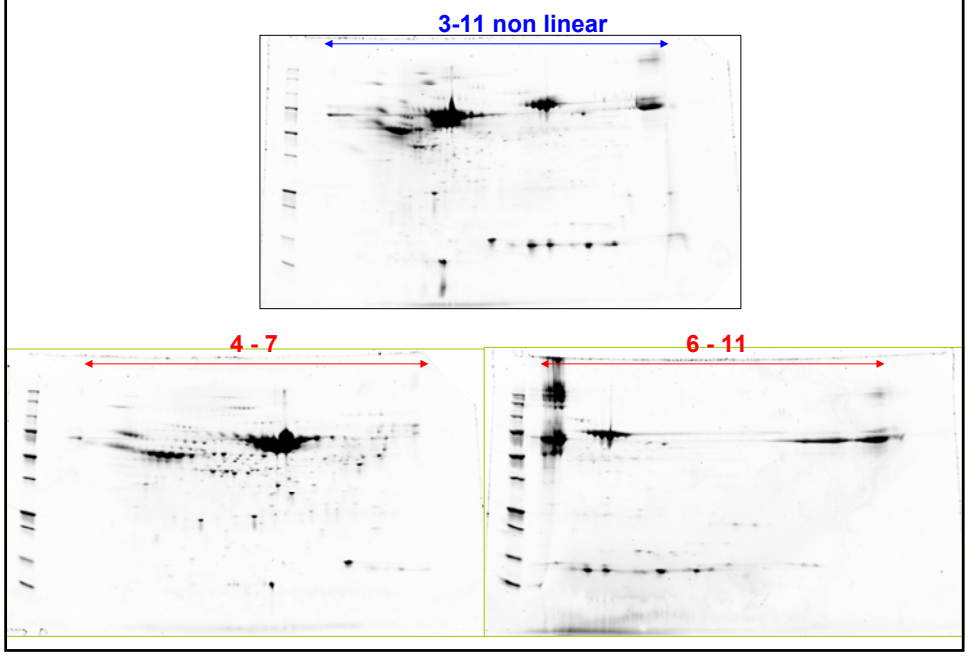
*** This protein list only represents spots observed in most of 9 gels. Detail analysis of differences between gels is not shown.**

UAB Center of Skin Diseases, ABRF 2006

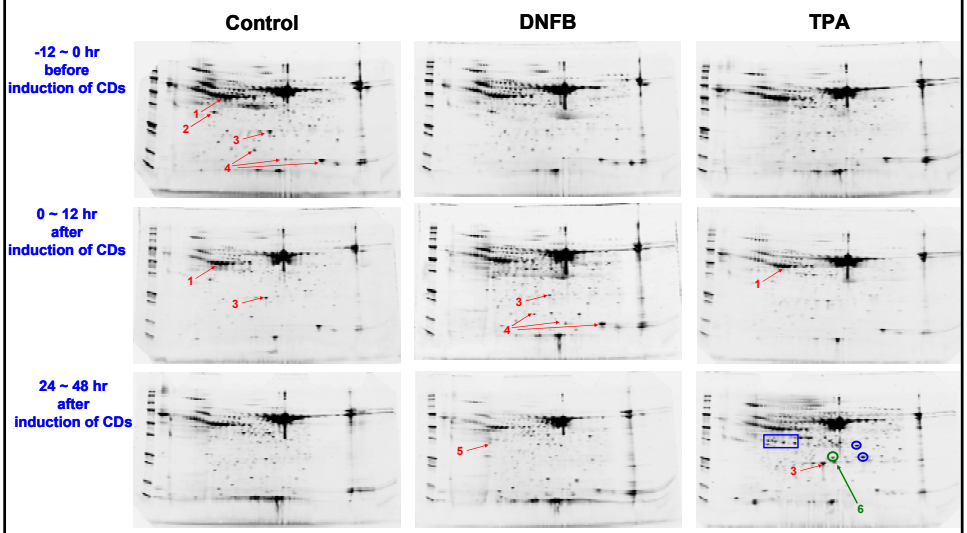
CUF for Proteomics Analysis of Interstitial Microenvironments

- We have evaluated the use of Capillary Ultrafiltration for proteomic study in interstitial microenvironments by providing both in-vivo and dynamic sampling.
- Challenges in analyzing CUF samples by 2DE:
  - Salty matrix: may not be a problem; desalt cleanup may lose proteins.
  - Albumin: albumin depletion assay: insufficient, protein loss.
  - Sample size: increase collection area (multiple probes or longer probes) and longer collection time (lost of temporal resolution).
  - Quantitative analysis: DIGE will help.

2DE Analysis of CUFs



2DE Analysis of CUFs from different CD Stages



60 μg, IEF: 4-7, 10-20 %% SDS PAGE, Sypro Ruby staining

Protein ID of IFs from different ACD Stages

Protein	Mass	MS	MOWSE	Spot
Cp Protein	121074	MALDI	126	
Gsn Protein	80712	MALDI	114	
Plasminogen	90723	MALDI	88	
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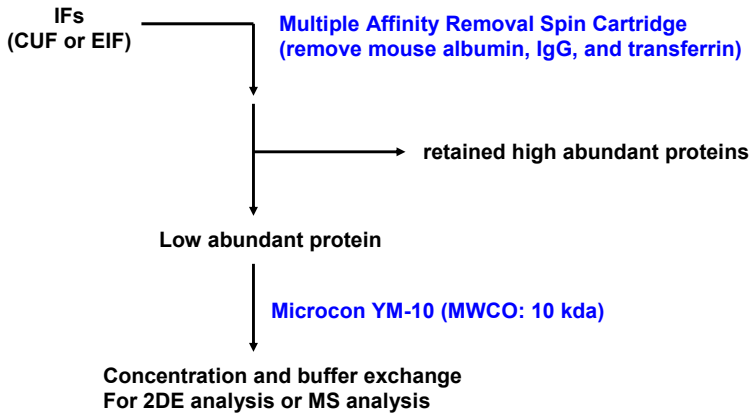
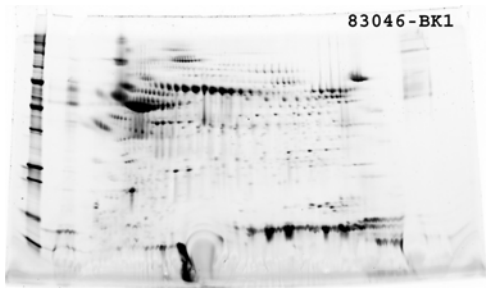
Apolipoprotein A-I,	30569	MALDI	118	
trophoblast specific protein-beta	13802	MALDI	66	
Vitamin D-binding protein	53085	Q-TOF		
Calgranulin B	12909	Q-TOF		
transthyretin	15766	MALDI	103	4
Apolipoprotein A-I, precursor	30358	MALDI	96	2
Apolipoprotein A-I	23008	MALDI	131	3
serine (or cysteine) proteinase inhibitor, clade A, member 1d	45969	MALDI	89	1
Serum amyloid P-component precursor	26230	MALDI	130	6
complement component c3d	33442	MALDI	74	5

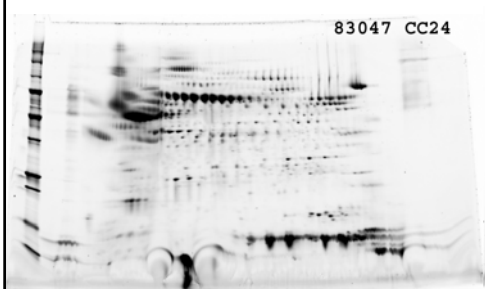
Sample Prep for Proteomic Analysis of IFs



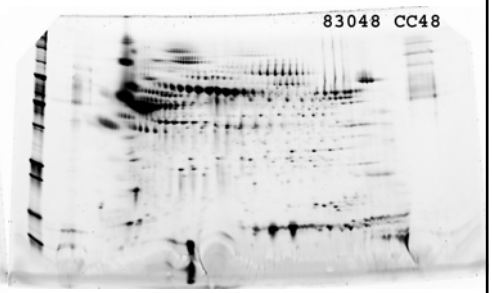
2DE Analysis of CUFs from different CD Stages



Pooled BK CUF collected -12-0 hr before treatment

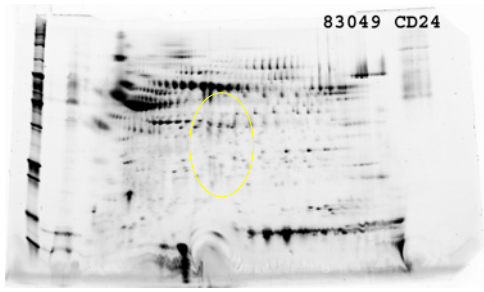


CC CUF collected 0-24 hrs after treatment

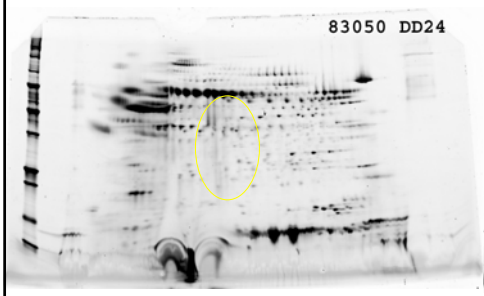


CC CUF collected 24-48 hrs after treatment

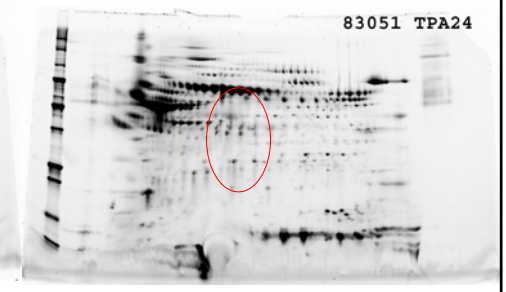
2DE Analysis of CUFs from different CD Stages



CD CUF collected 0-24 hrs after DUFB treatment



ACD (DD) CUF collected 0-24 hrs after treatment



Irritation CUF collected 0-24 hrs after TPA treatment

2DE Analysis of TIFs from TPA or DNFB Irritated Ear

Male C3H/HeN mice

↓

Irriration induced by painting
1 % DNFB or 20 nmol TPA on ears

↓ 6 hours

Harvest dorsal ear skin,
let the ear skin float on PBS with PI (1ml/ear),
and incubate at 37 C for 1 ½ hour

↓

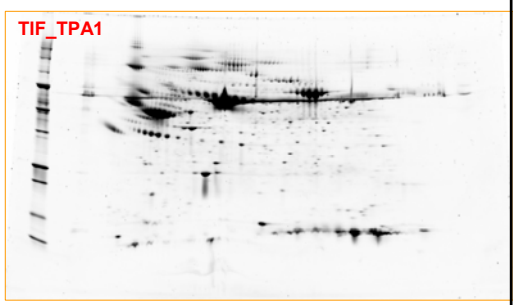
Collect supernatant as TIF

↓ PPT with 2D cleanup kit

Dissolved in IEF buffer
for 2DE analysis

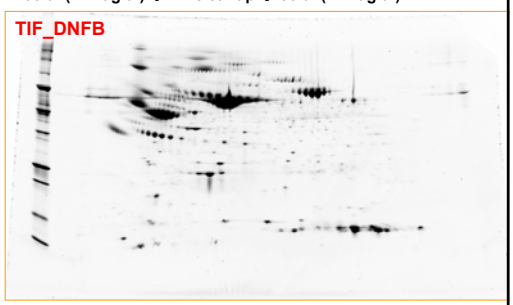
60 ug
3-11NL + 10-20% gel

TIF_TPA1



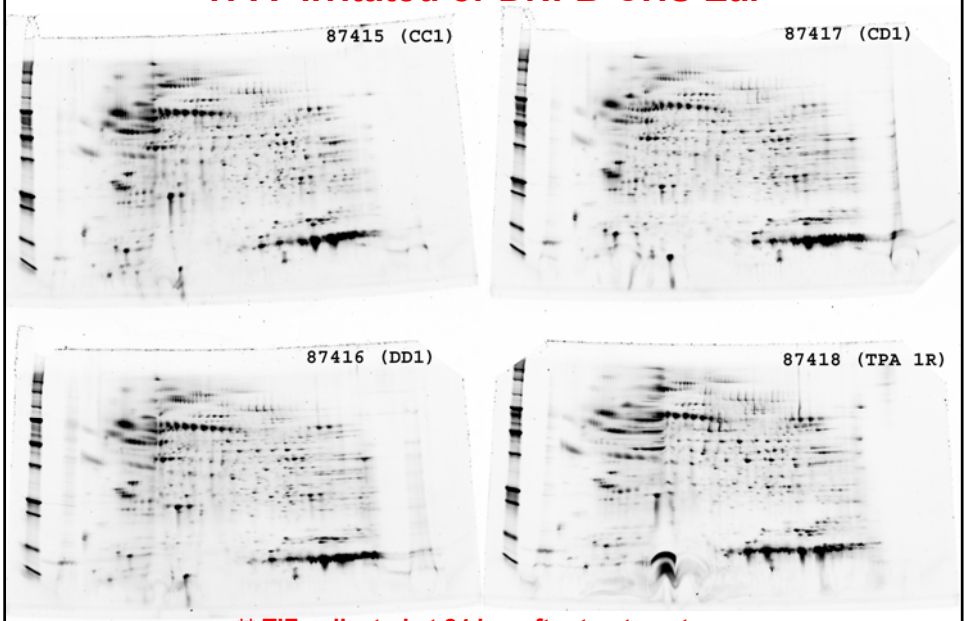
100 ul (1.12ug/ul) → 2D cleanup → 50 ul (2.11ug/ul)

TIF_DNFB



100 ul (0.95ug/ul) → 2D cleanup → 50 ul (1.81ug/ul)

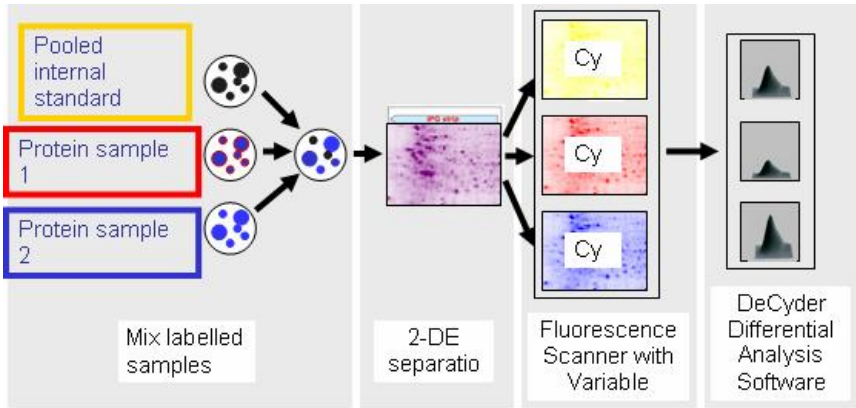
2DE Analysis of TIFs Collected from TPA- Irritated or DNFB-CHS Ear



** TIF collected at 24 hrs after treatment

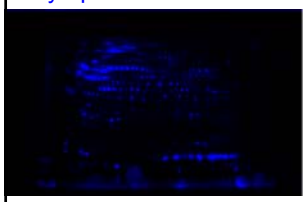
Quantitative Proteomics

- Gel_based approach:
  - **Difference gel electrophoresis (DIGE)**

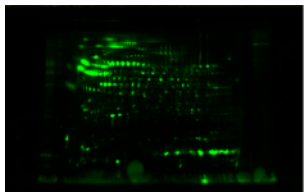


DIGE of TIF samples

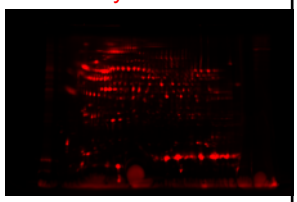
Cy2-pooled standard



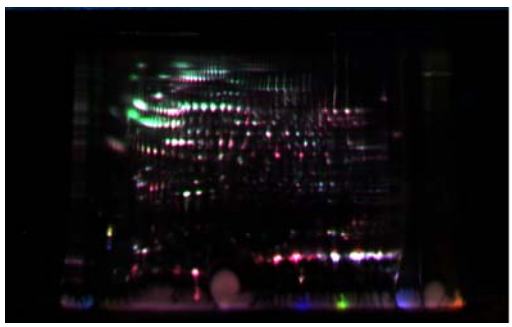
Cy3-TIF DD



Cy5-TIF TPA



- 10 µg/ Cy dye labeled samples
- limited cy dye labeling (0.5%)
- 30 mg/gel
- IEF: 3-11 NL; 10-20% SDS PAGE



Gel 2 (84174, DD-TPA) Cy2 Cy3 Cy5 Overlay

Summary

- **The use of Capillary Ultrafiltration for proteomic study in interstitial microenvironments by providing both in-vivo and dynamic sampling.**
- **Challenges in analyzing UF samples by 2DE:**
  - **Salty matrix: may not be a problem; desalt cleanup may lose proteins.**
  - **Albumin: depletion kit from Sigma did not work well.**
  - **Sample size: increase collection area (multiple probes) and longer collection time (lost of temporal resolution).**
  - **One shot deal: DIGE will help.**
- **Other Multi-dimentional approaches may be more suitable for analysis of IF samples.**