Elucidating the Pharmacology and Toxicology of Anti-HIV Drug Metabolites

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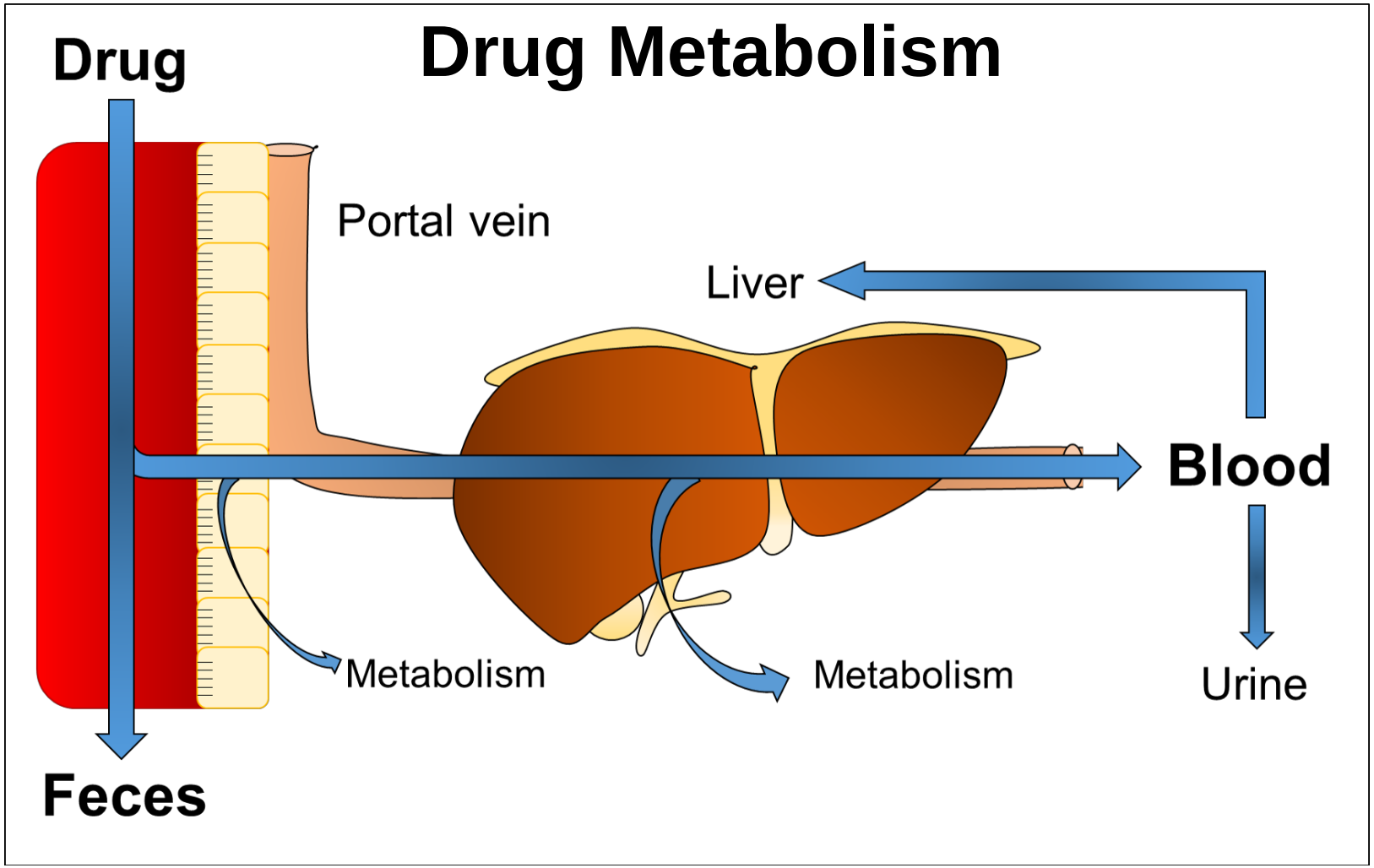


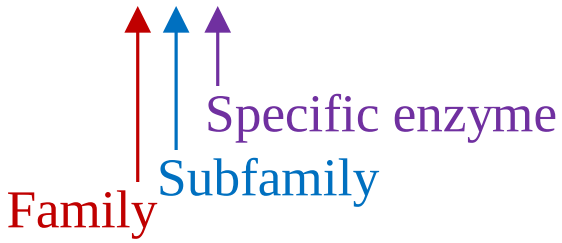
- Drugs tend to be lipophilic
- Metabolism generates more polar products that can be excreted readily

The Cytochromes P450 (CYP)

- A superfamily of heme-containing enzymes
- Metabolize drugs and endogenous molecules
- The general oxidation reaction

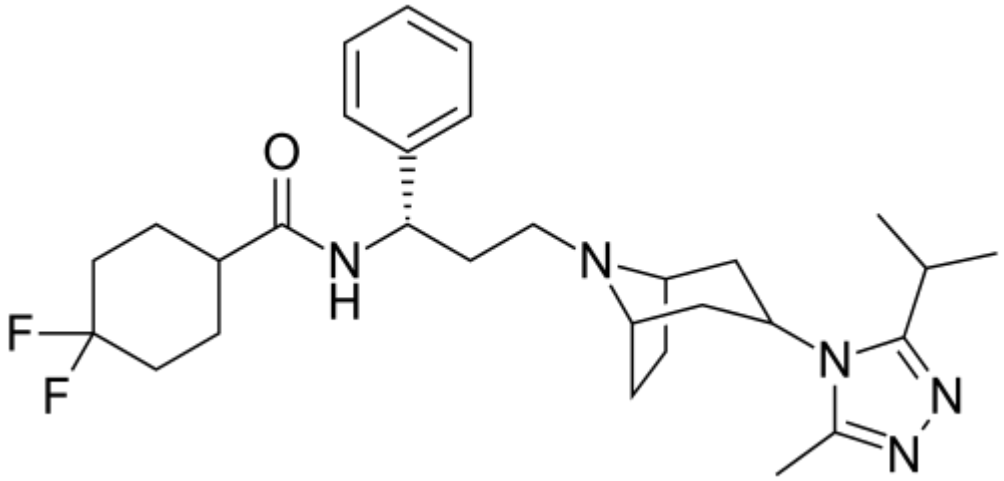
$$NADPH + H^+ + O_2 + R \rightarrow NADP^+ + H_2O + RO$$

- Nomenclature
  - Example: CYP3A4



Maraviroc

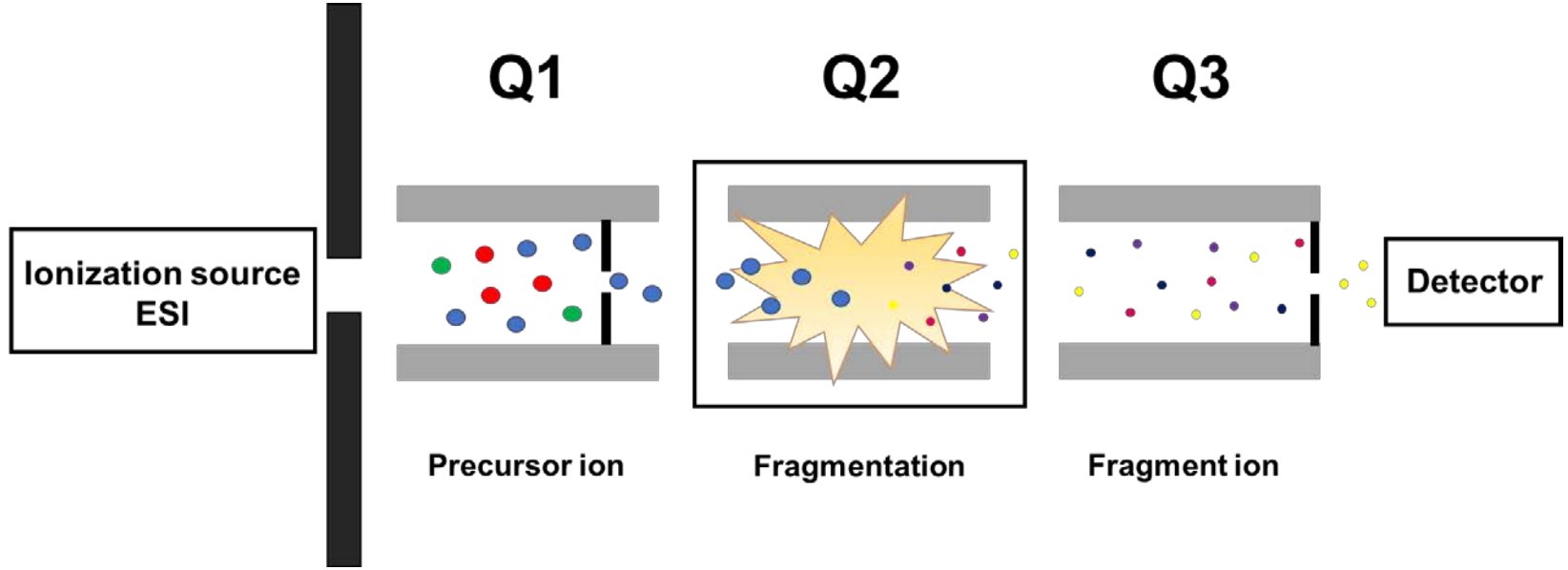
- Used in the treatment of HIV infection
- A chemokine receptor CCR5 antagonist
- A candidate for HIV prevention



Separation and Detection of Metabolites: LC-MS/MS

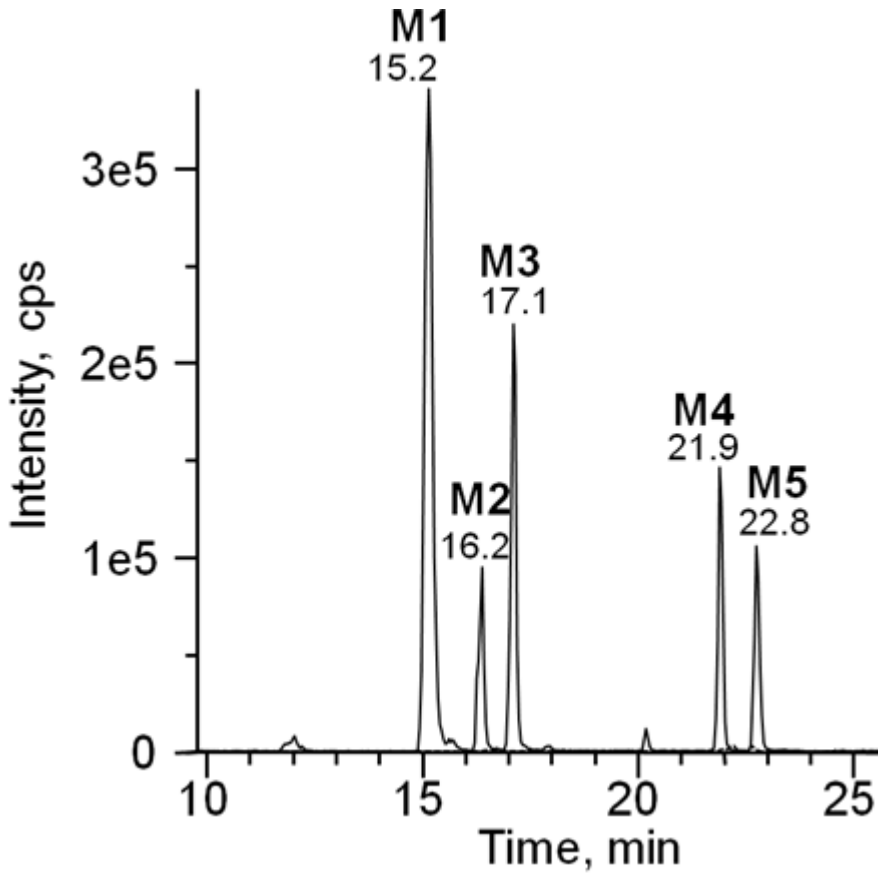
uHPLC

Triple Quad Mass Spectrometry

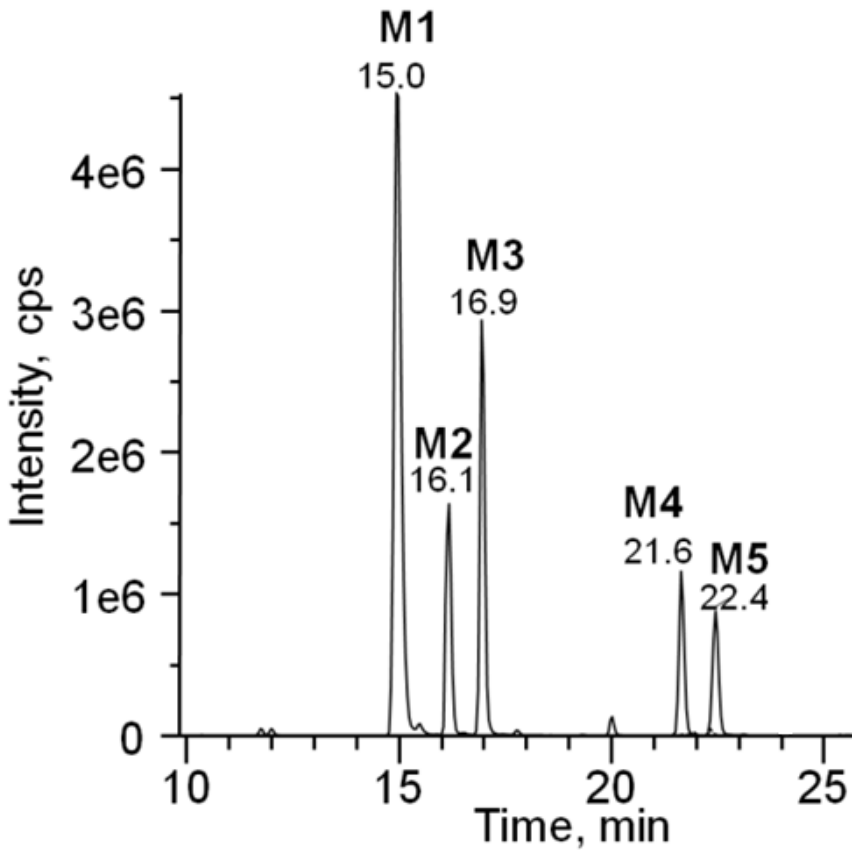


Separation of Oxidative Metabolites of Maraviroc

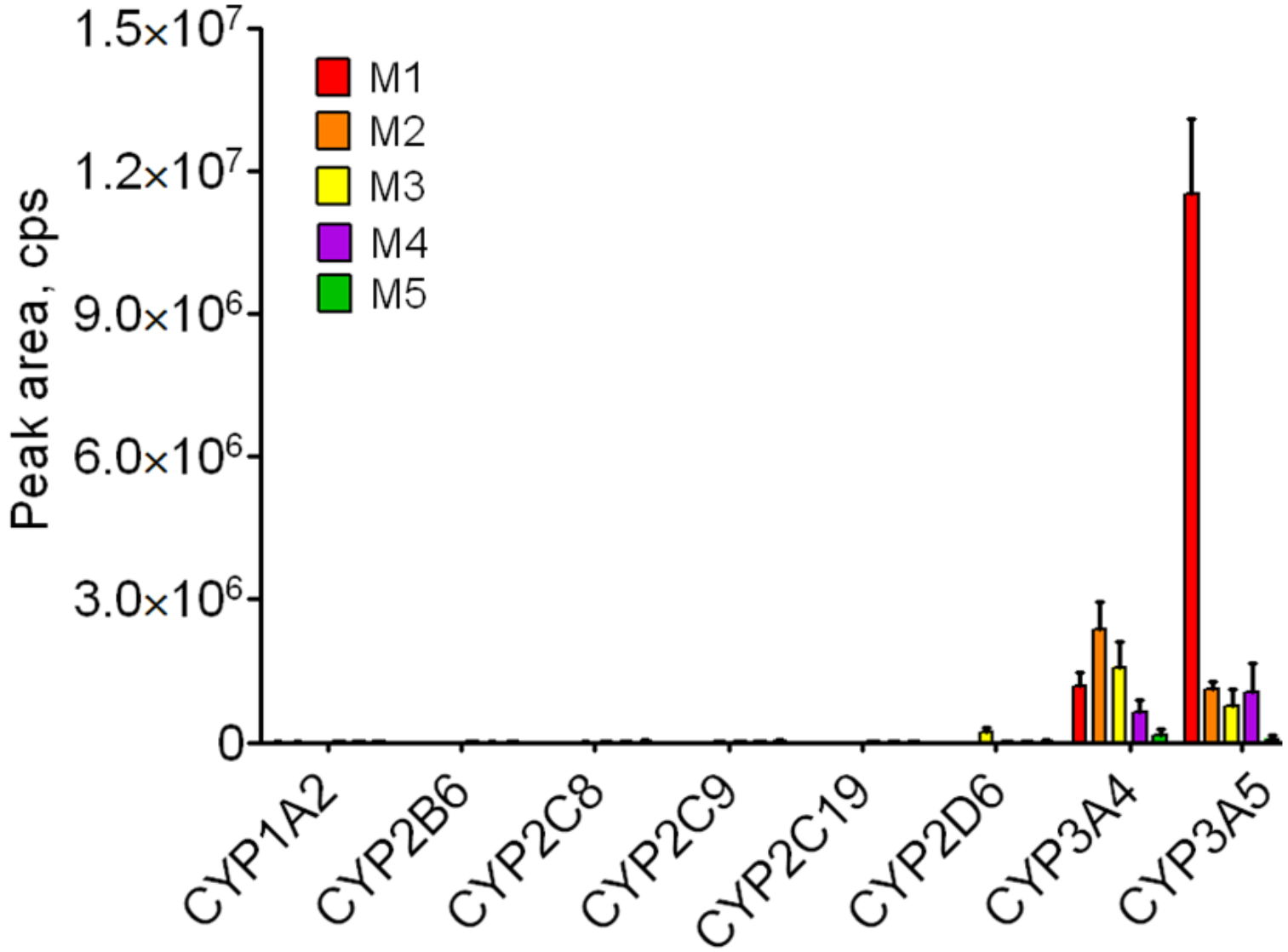
Human Plasma



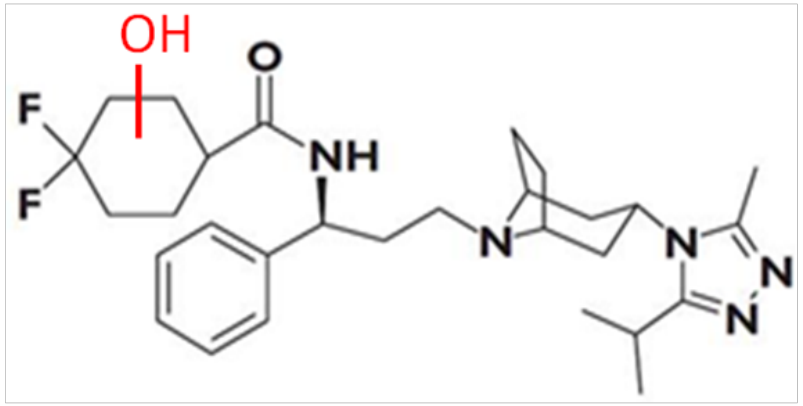
Human Urine



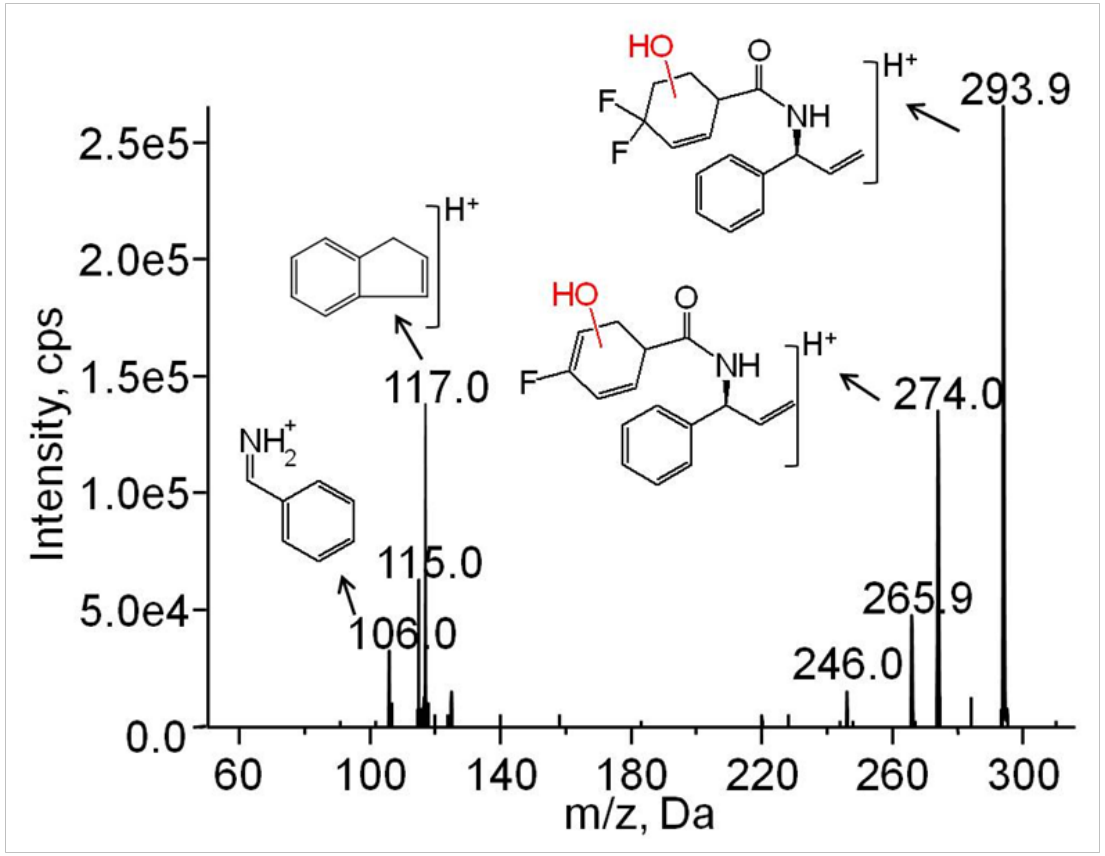
CYP34 and CYP3A5 Metabolize Maraviroc



M1 Results from Oxygen Insertion on the Difluorocylcohexane Ring of Maraviroc



Proposed M1 structure



Divergent CYP3A4 and CYP3A5 Residues

```
3A4  54  HKGFCMF 60
          +G   F
3A5  54  RQGLWKF 60
```

Substrate Recognition Site (SRS1)

```
3A4  101 VFTNRRPFGPVGFMKSAISIAED123
         VFTNRR GPVGFMKSAIS+AED
3A5  101 VFTNRRSLGPVGFMKSAISLAED123
```

SRS2

```
3A4  204 VENTKKLLRFDFL 216
         VE+TKK L+F  FL
3A5  204 VESTKKFLKFGFL 216
```

SRS3

```
3A4  238 ICVFPRE 244
         ++ FP++
3A5  238 VSLFPKD244
```

SRS4

```
3A4  294 ELVAQSIIFIFAGYETTSS 312
         EL AQSIIFIFAGYETTSS
3A5  294 ELAAQSIIFIFAGYETTSS 312
```

SRS5

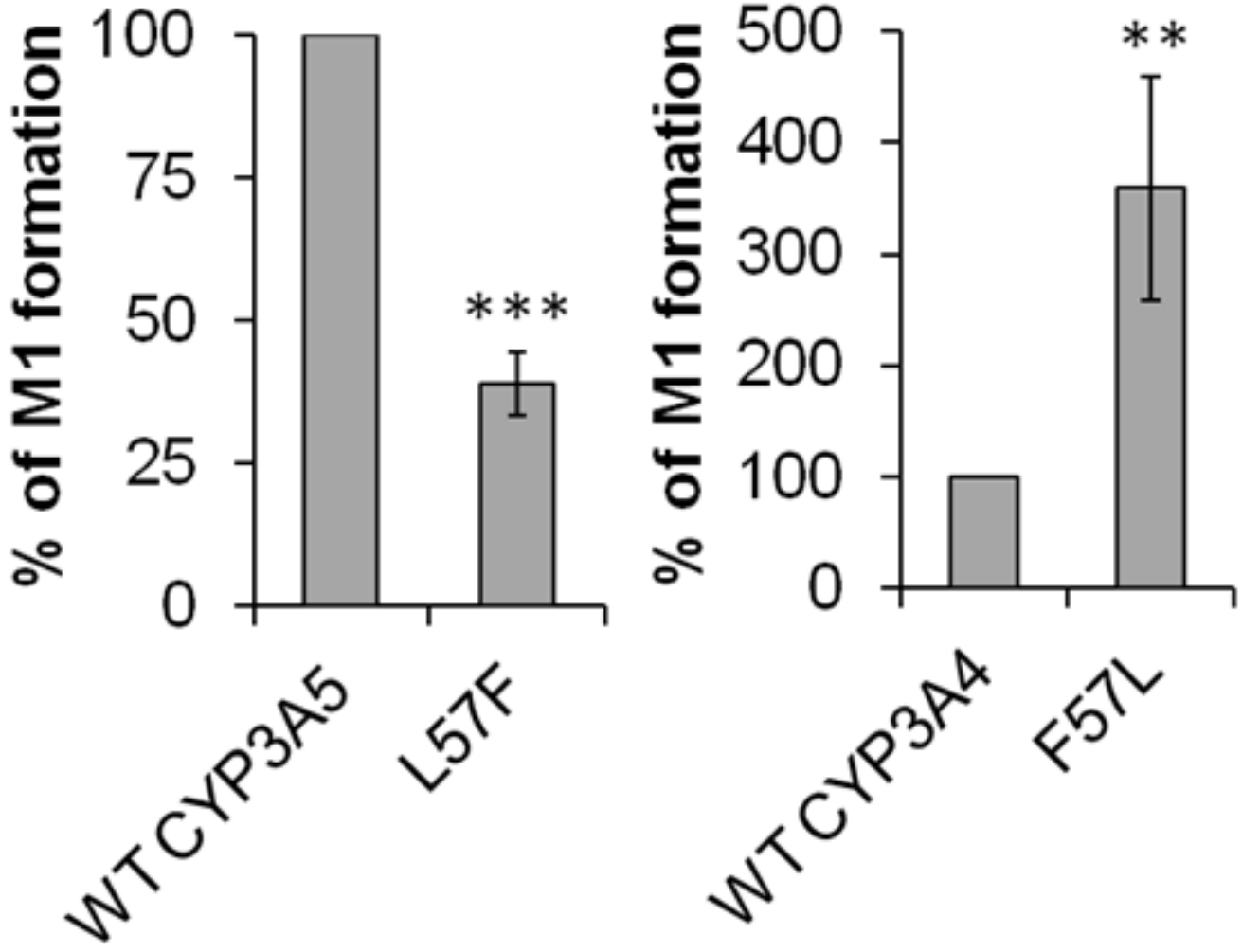
```
3A4  368 PIAMRLERV 376
         P+A +RLER
3A5  368 PVA IRLERT 376
```

SRS6

```
3A4  476 KLSLG GLLQP 485
         KL   GLLQP
3A5  475 KLDTQGLLQP 484
```


Biochemistry 1998; 37:12536-45
J Biol Chem 2004; 279:38091-4
Xenobiotica 2006; 36:219-33
Curr Drug Metab 2011;12:684-700

Residue 57 Differentiates CYP3A4 vs. CYP3A5 M1 Formation



Study Design

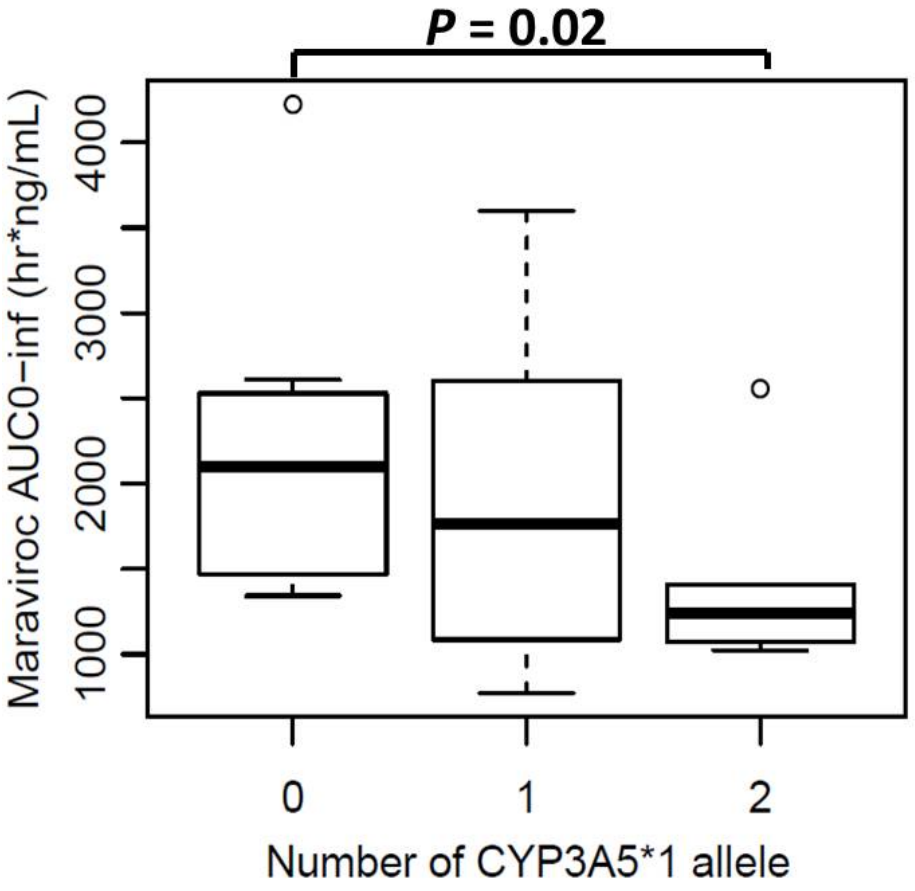
- **Twenty four healthy volunteers** (N=8 in each group)
  - Homozygous *1 (two *1 alleles)
  - Heterozygous (one *1 allele and one variant allele*3, *6, or *7)
  - Dysfunctional (two variant alleles)

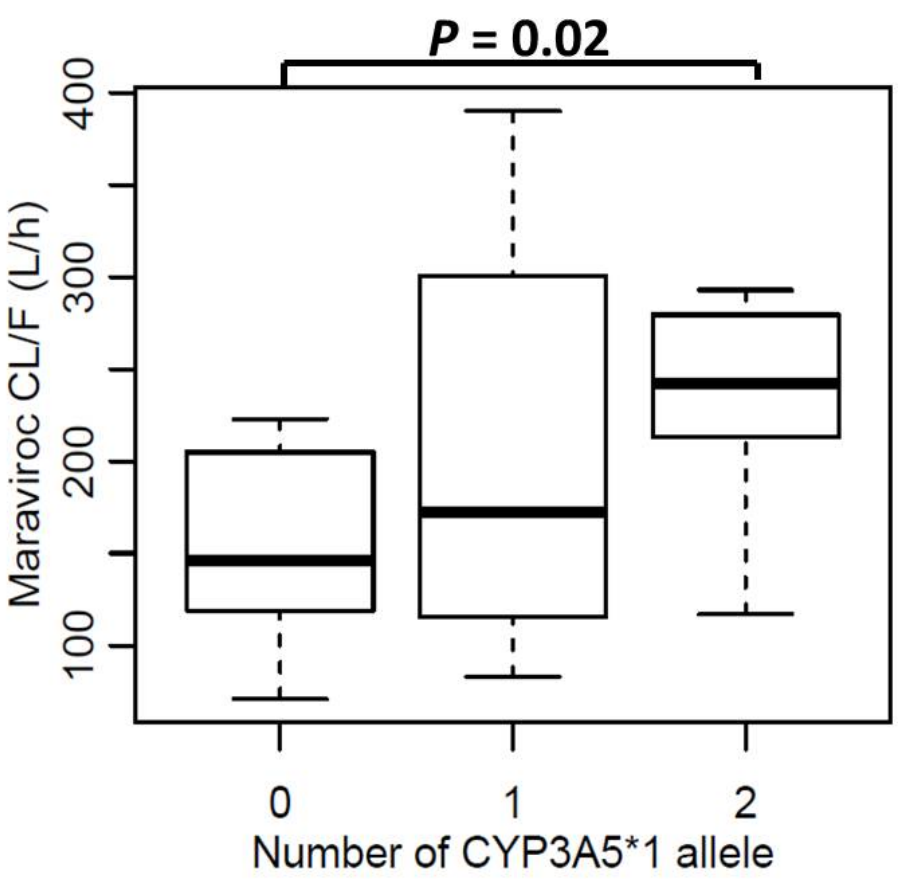
- **Treatment**: a single oral dose of 300 mg maraviroc

- **Blood collection**: predose and 1, 2, 3, 4, 6, 8, 10, 24 and 32 h after the dose

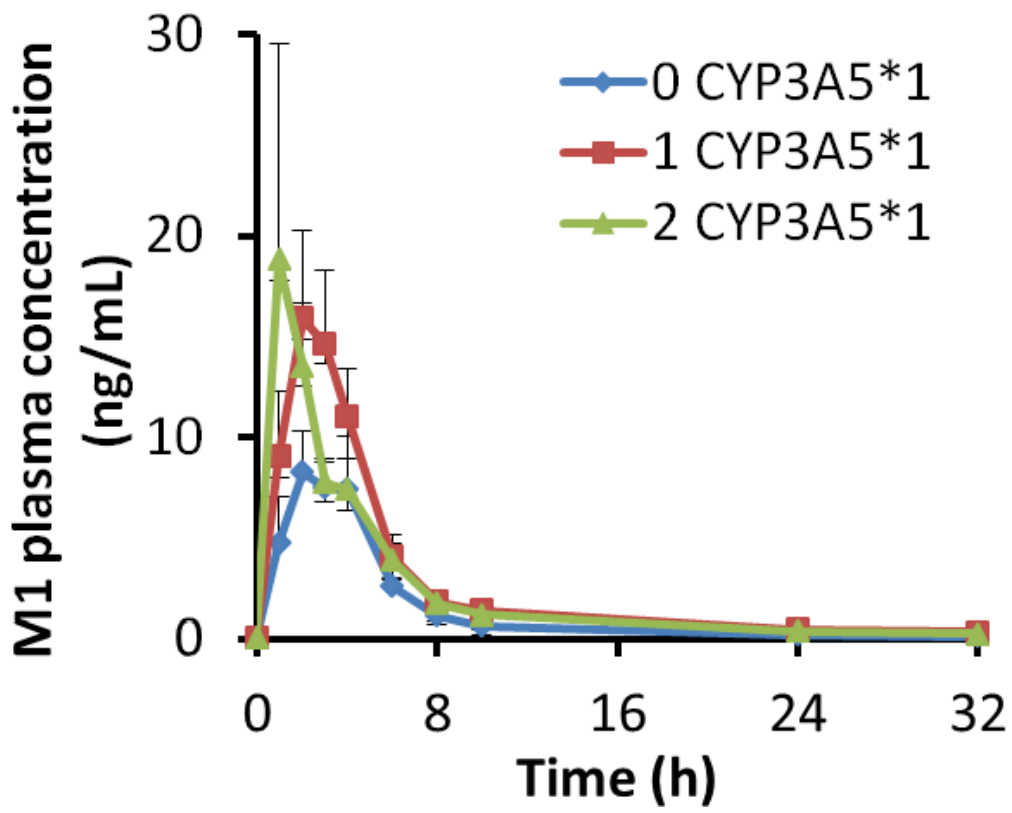
- **Concentration measurement**
  - LC-MS/MS

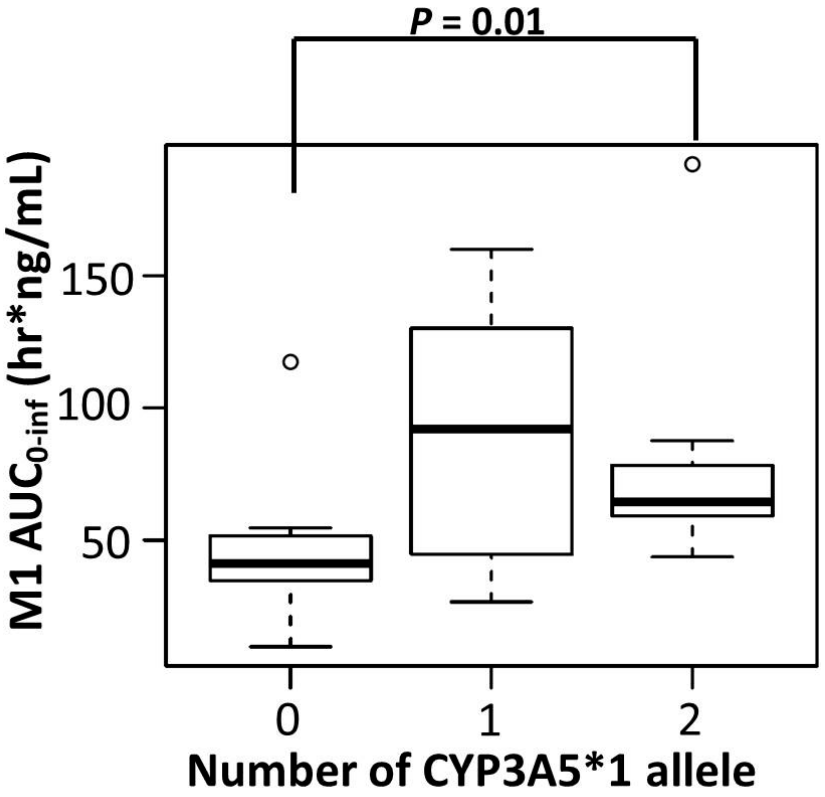
Decreased Maraviroc Exposure and Increased Clearance in the CYP3A5 Homozygous *1 Group



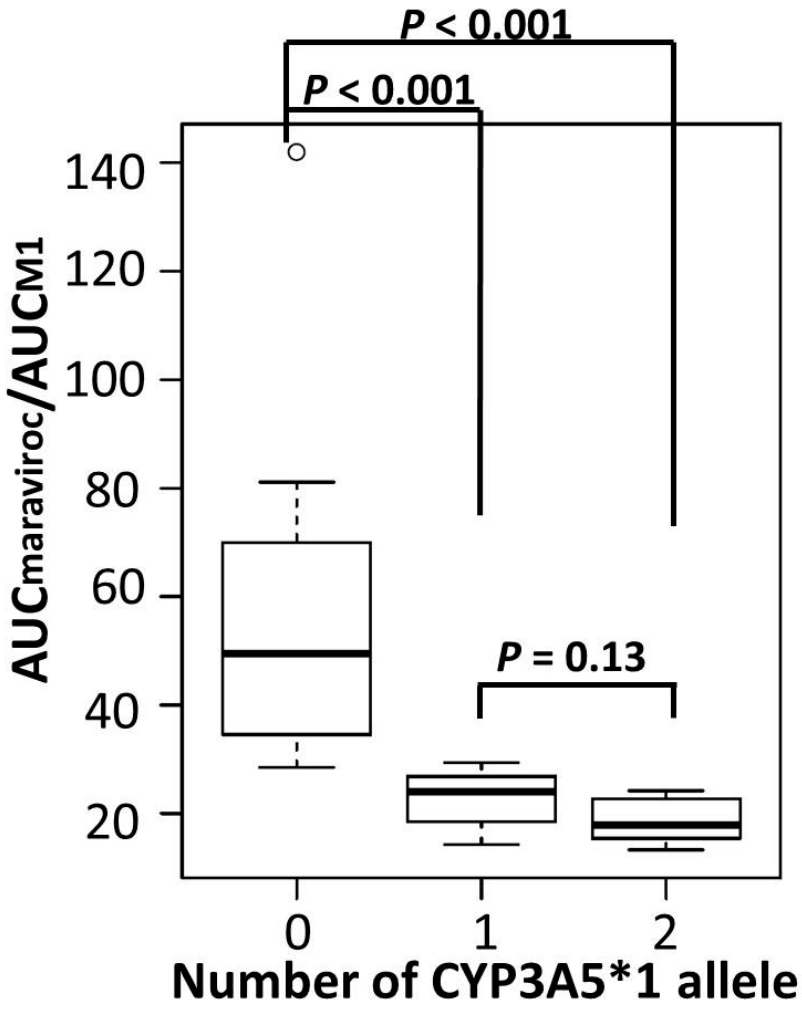


Decreased M1 Formation by CYP3A5 Dysfunctional Group

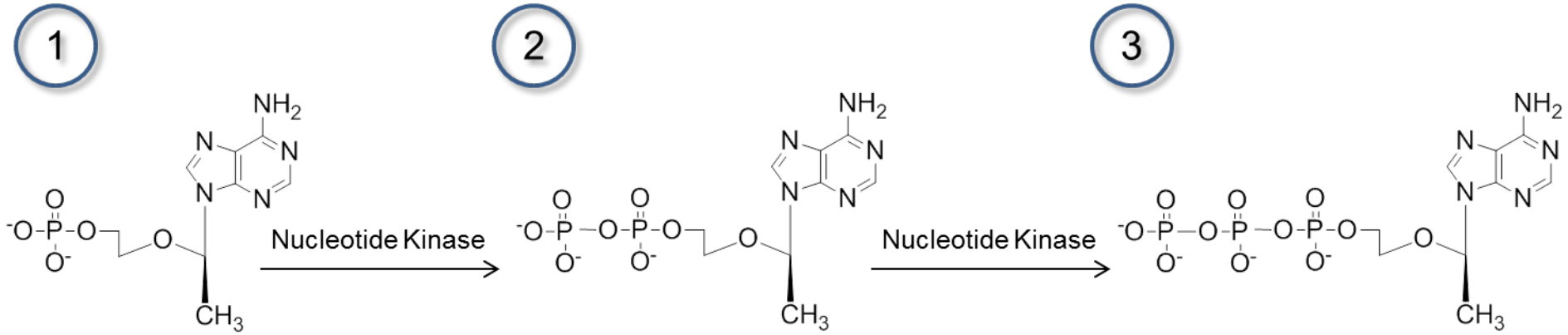




Lower AUC Ratio (maraviroc/M1) in CYP3A5*1 Allele Carriers



Tenofovir is a Nucleotide Reverse Transcriptase Inhibitor



Tenofovir
(TFV)

TFV Monophosphate
(TFV-MP)

*TFV Diphosphate
(TFV-DP)

Candidate Nucleotide Kinases

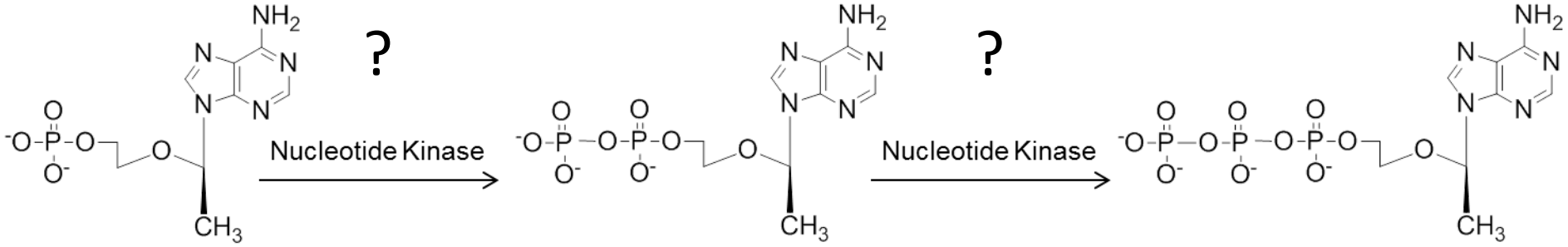
Adenylate kinase 2 (AK2)

Guanylate kinase 1 (GUK1)

Creatine kinase, muscle (CKM)

Pyruvate kinase, muscle (PKM)

Pyruvate kinase, liver & RBC (PKLR)



Tenofovir
(TFV)

TFV Monophosphate
(TFV-MP)

TFV Diphosphate
(TFV-DP)

siRNA Knockdown of Nucleotide Kinases

Human Samples

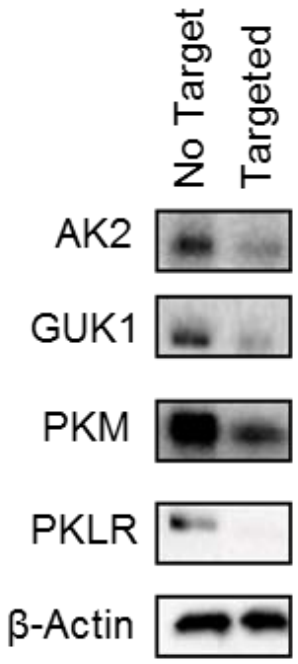
- Cells and tissues:

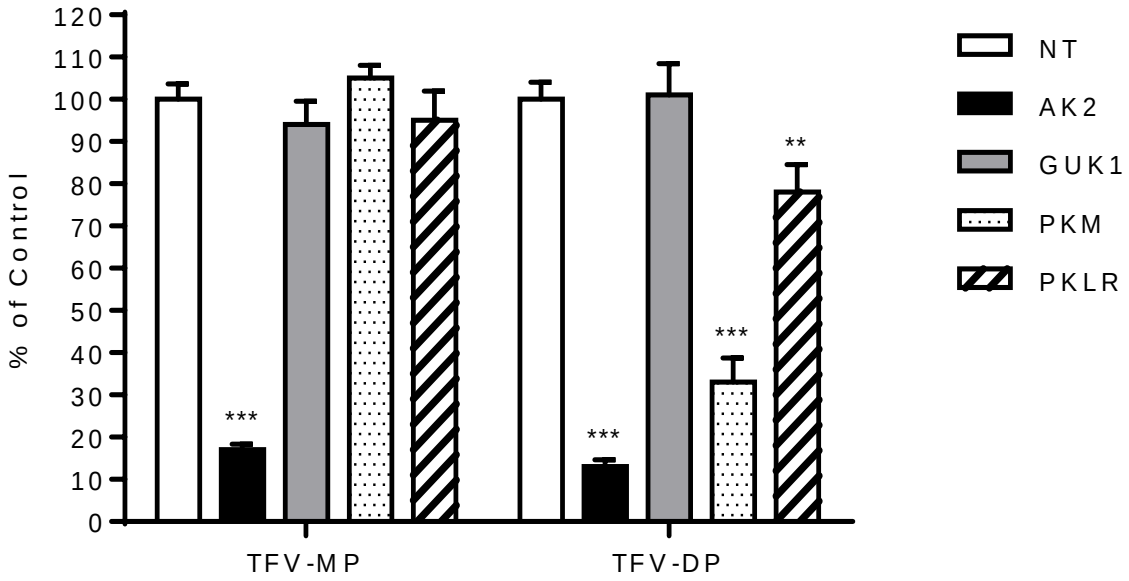
1. Peripheral blood mononuclear cells (PBMC)
2. Colorectal tissue
3. Vaginal tissue

Method

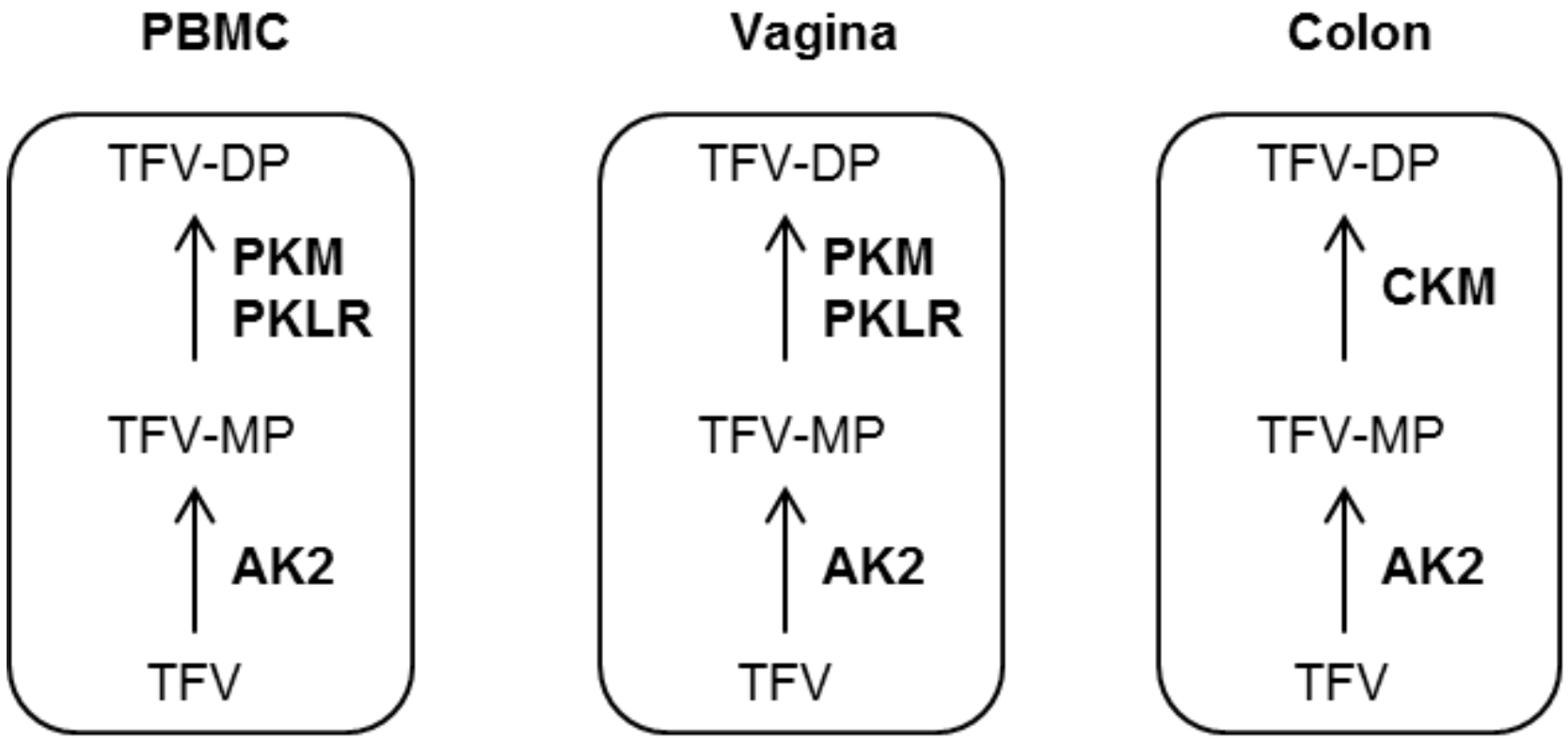
- Delivered siRNA to cells and tissues in culture
- Followed by incubation with TFV
  - Detected TFV metabolites using ultra-high performance liquid chromatography-tandem mass spectrometry

AK2, PKM, and PKLR Contribute to Metabolite Formation in Peripheral Blood Mononuclear Cells (PBMC)



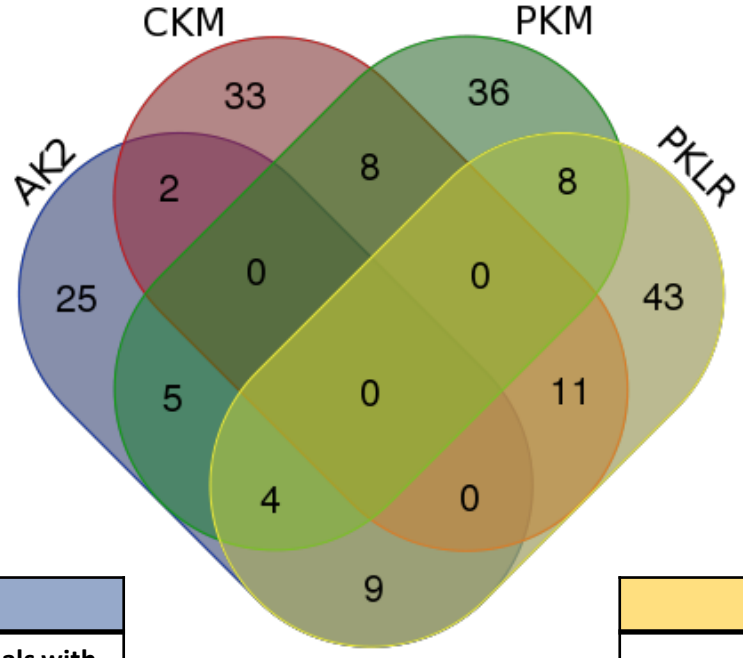


Tenofovir is Activated in a Tissue-Specific Manner



Creatine Kinase, Muscle (CKM)			
Country	**Individuals Sequenced**	**Individuals with one or more variant**	**% of individuals with one or more variant**
United States	582	23	4%
South Africa	231	17	7%
Uganda	24	5	21%
Thailand	171	9	5%
Total	**1,008**	**54**	**5%**

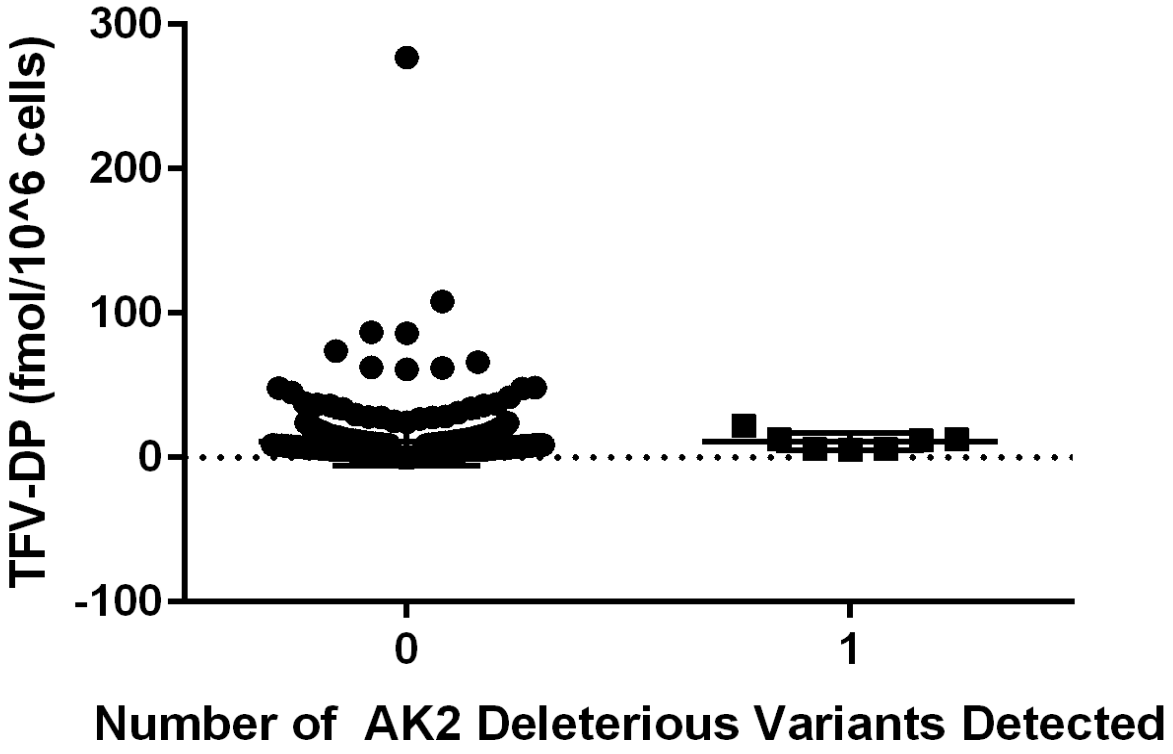
Pyruvate Kinase, Muscle (PKM)			
Country	**Individuals Sequenced**	**Individuals with one or more genetic variant**	**% of individuals with one or more variant**
United States	582	30	5%
South Africa	231	15	6%
Uganda	24	5	21%
Thailand	171	11	6%
Total	**1,008**	**61**	**6%**



Adenylate Kinase, 2 (AK2)			
Country	**Individuals Sequenced**	**Individuals with one or more genetic variant**	**% of individuals with one or more variant**
United States	582	33	6%
South Africa	231	7	3%
Uganda	24	3	12.5%
Thailand	171	2	1%
Total	**1,008**	**45**	**4%**

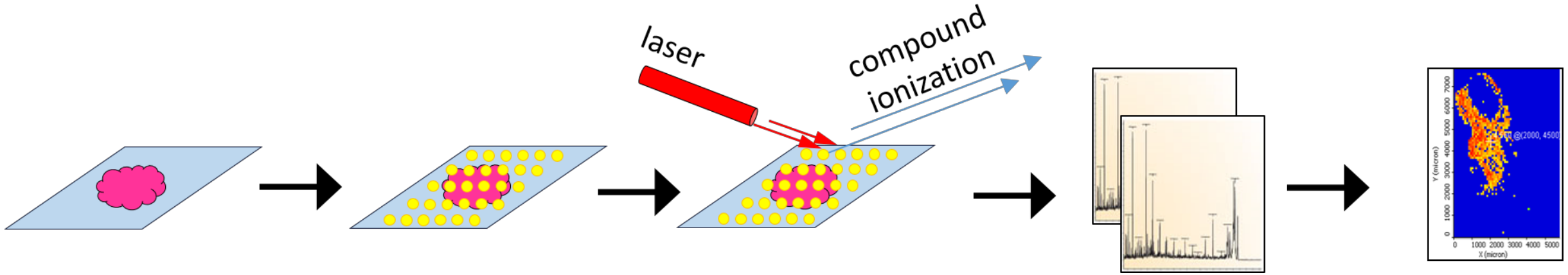
Pyruvate Kinase, Liver and Red Blood Cell (PKLR)			
Country	**Individuals Sequenced**	**Individuals with one or more genetic variant**	**% of individuals with one or more variant**
United States	582	46	8%
South Africa	231	16	7%
Uganda	24	3	12.5%
Thailand	171	10	6%
Total	**1,008**	**75**	**7%**

Tenofovir Diphosphate Levels In Genotyped Individuals

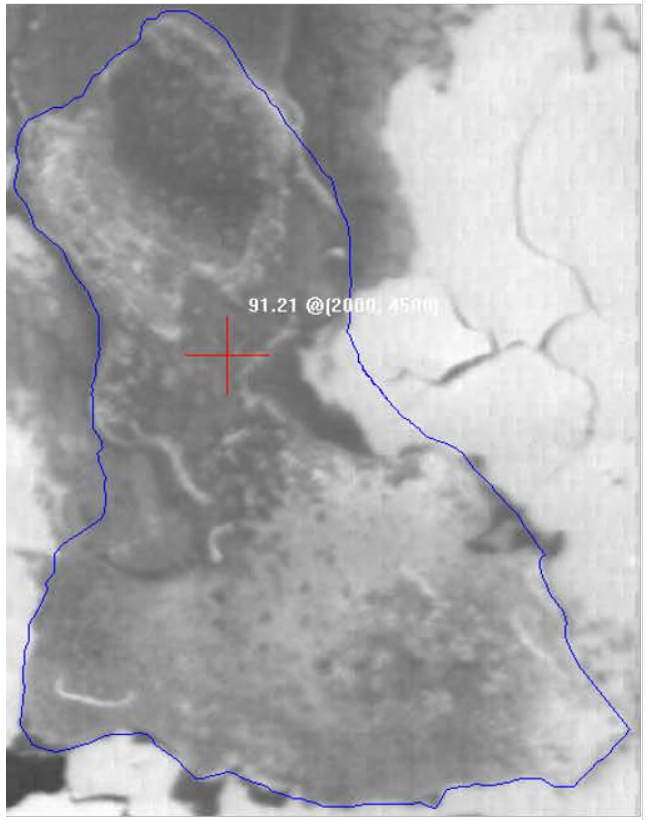


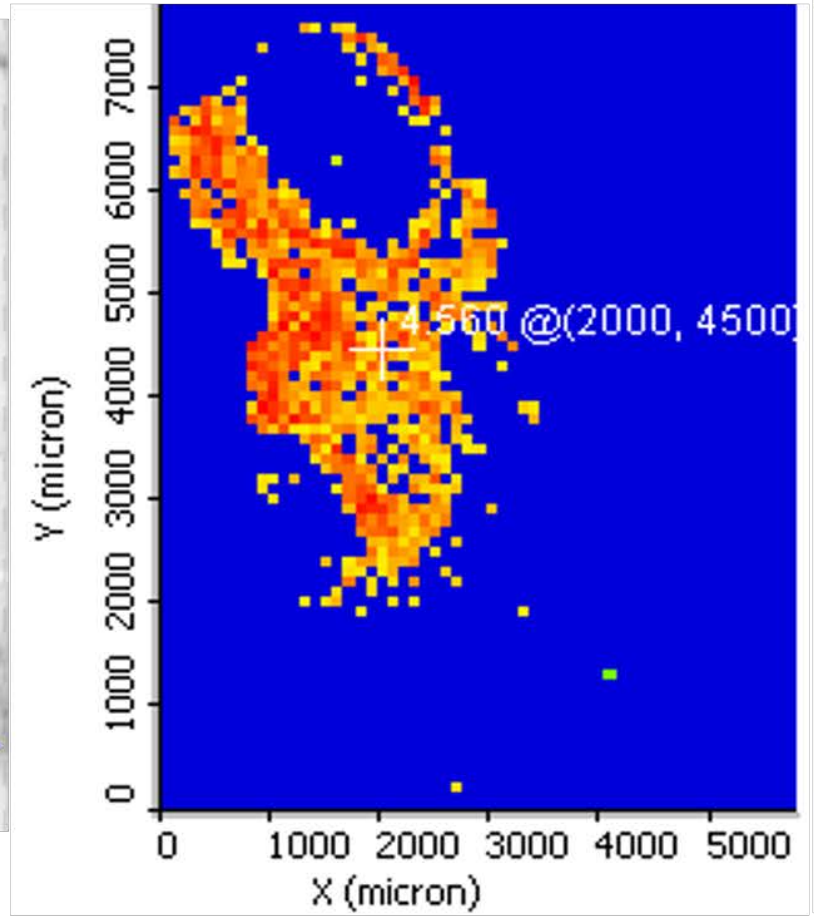
Individuals with no Predicted AK2 Deleterious Variants Detected	477
Individuals with Predicted AK2 Deleterious Variants Detected	7

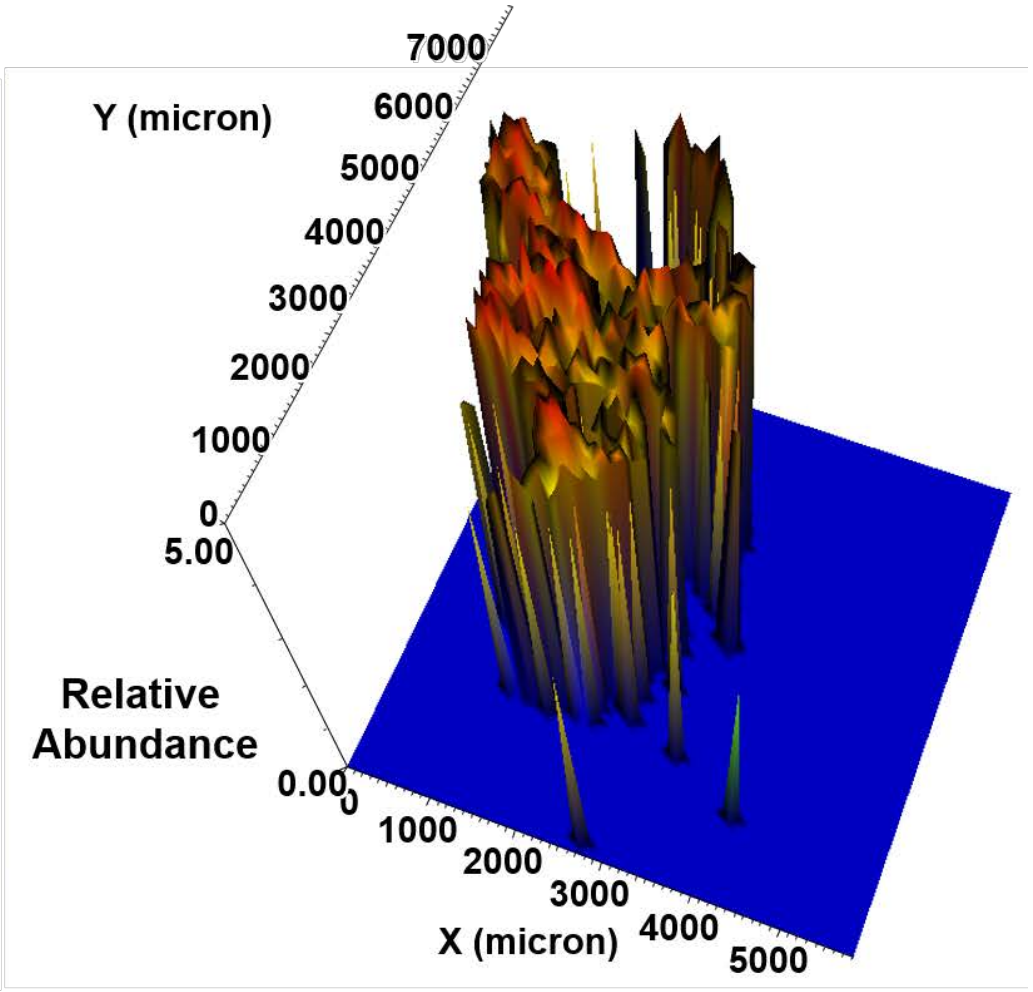
Matrix-assisted laser desorption/ionization coupled to mass spectrometry (MALDI-MS) for understanding spatial distribution

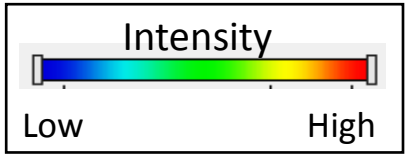


MALDI-MS/MS Reveals Heterogeneity in TFV distribution within Cervical Tissue









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