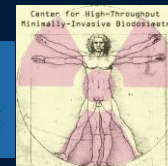


Rapid biodosimetry in biofluids: targeted approaches through small molecules

Evagelia (Lia) C. Laiakis, Ph.D.

Associate Professor

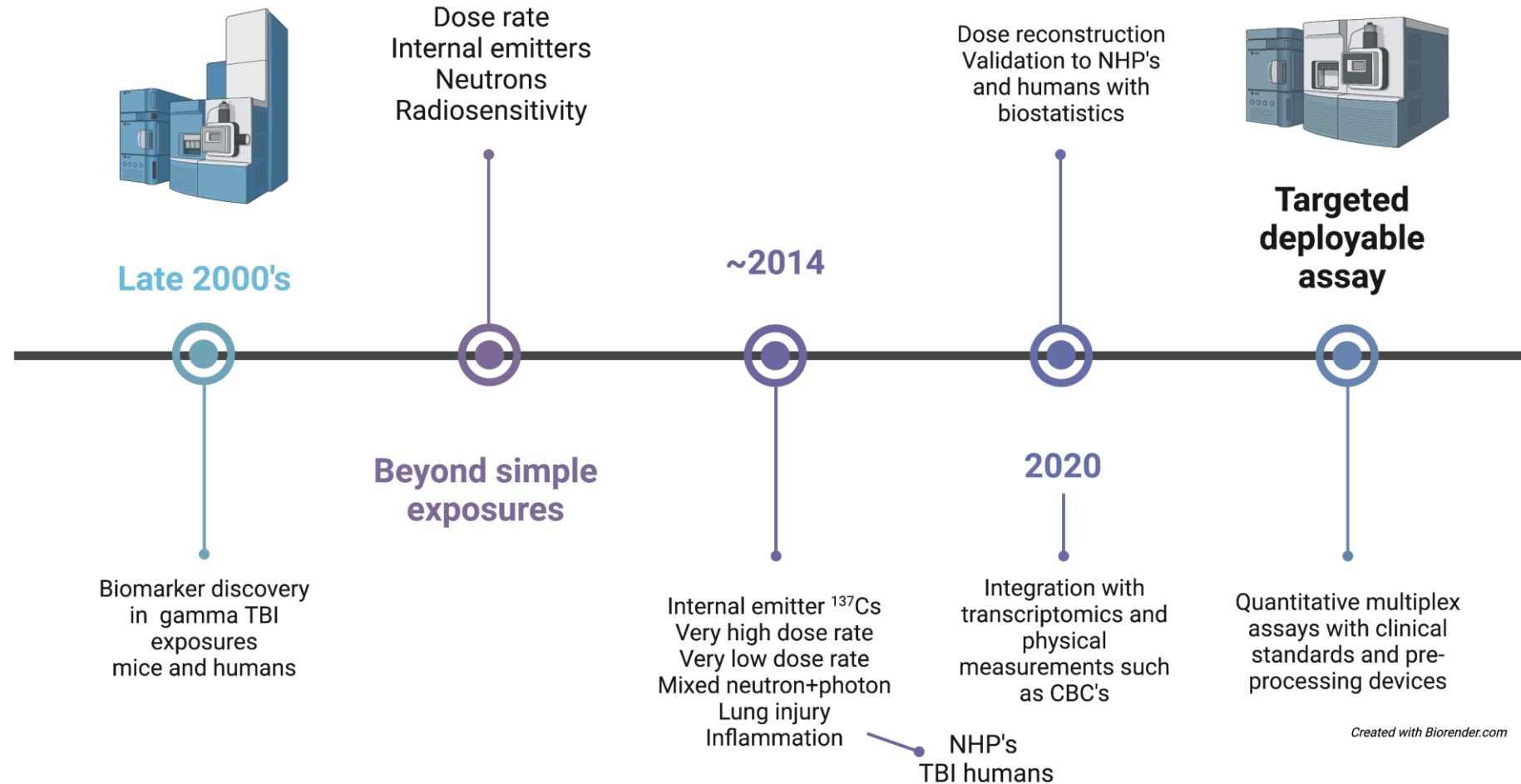
Department of Oncology, Lombardi Comprehensive Cancer Center
Department of Biochemistry and Molecular & Cellular Biology



GEORGETOWN UNIVERSITY

Biodosimetry through small molecules

Radiation Metabolomics - a timeline




X-rays or γ -rays
Neutrons, mixed fields




≤ 7 days

> 7 days and DEARE

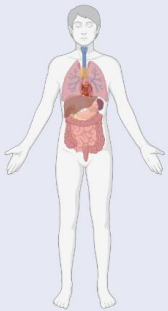
1



Mice



NHPs



Humans

2

Urine



Serum

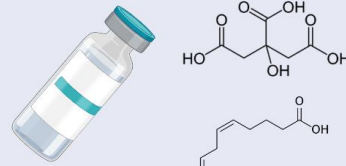


Saliva

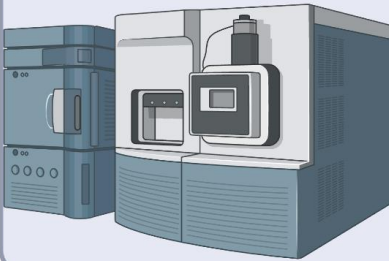


3

Liquid extraction

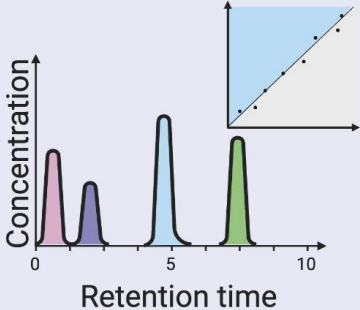


Liquid chromatography mass spectrometry



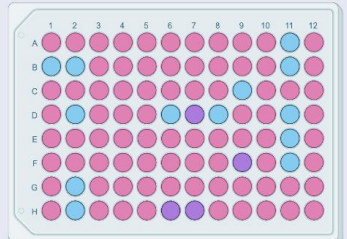
4

Multiplex panels



5

Pre-processing technology



Mice				Nonhuman primates (NHPs)			
Urine		Serum		Urine		Serum	
Increased	Decreased	Increased	Decreased	Increased	Decreased	Increased	Decreased
Citrate	a-Ketoglutarate	Carnitine	Citrulline	Carnitine	Xanthine	Leucine	Serine
Taurine	Succinate	L-Alanine	Fumarate	Acetylcarnitine	Glycine	Isoleucine	Alanine
Uric acid	Citrate	Glutamic acid	Sphinganine	Butyrylcarnitine	Methylsuccinic acid	Arginine	Asparagine
Nicotinate	Hippuric acid	Malic acid	9-HOTrE	Taurine	Phenylacetic acid	LysoPC(20:4)	Valine
Xanthine	2-Oxoglutaric acid	Riboflavin	5-HEPE	Cortisone	Citric acid	LysoPC(22:6)	Proline
Xanthosine	4-Pyridoxic acid	Sphingosine	14,15-DiHETE	Cortisol	Malic acid	PC(16:0/22:6)	Tyrosine
Allantoin	Kynurenic acid	Sphingosine-1P	17,18-DiHETE	Hypoxanthine	<i>cis</i> -Aconitic acid	SM(18:2/18:0)	Hypoxanthine
Hexanoylglycine	Xanthurenic acid	Ceramide	Oleic acid	Xanthosine	Succinic acid	Linoleic acid	Taurine
Glutaric acid	Pantothenic acid	Sphingomyelin	alpha-Linolenic acid	Xanthurenic acid	Isocitric acid	Arachidonic acid	Lysine
Acetylcarnitine	Inosine	GlcCer(d18:1/24:1)	Linoleic acid	Kynurenic acid	Lactic acid	Carnitine	Aspartic acid
Deoxyuridine	L-Arginine	GlcCer(d18:1/16:0)		Creatine	Palmitic acid	Propionylcarnitine	Threonine
Uridine	Glutamine	TXB2			Propionylglycine	Acetylcarnitine	PC(18:0/20:2)
		PGF2a			Hippuric acid	Butyrylcarnitine	LysoPC(14:0)
		12-HETE				Valerylcarntine	Betaine
		8-HETE				Xanthine	Tetradecadienylcarnitine
		Arachidonic acid				Creatine	Octadecadienylcarnitine

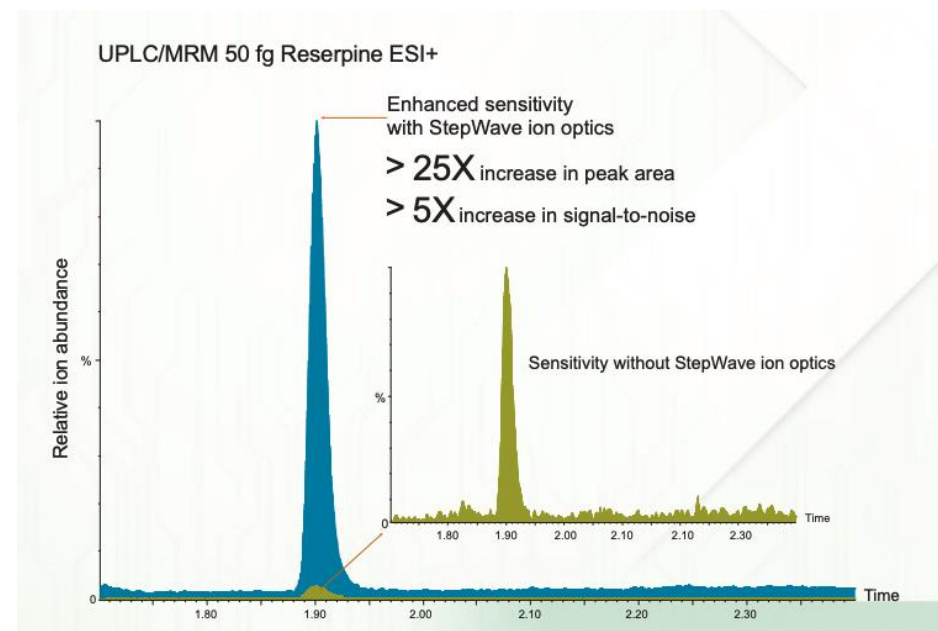
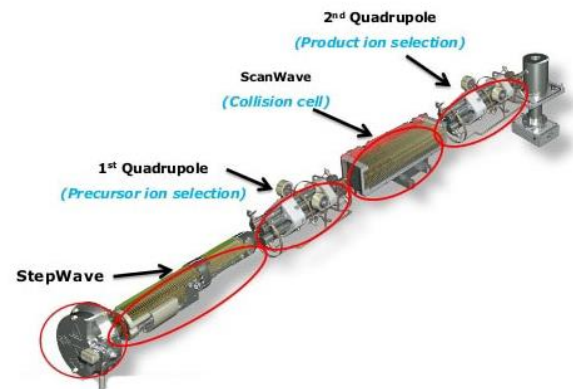
Human Patients			
Urine		Serum	
Increased	Decreased	Increased	Decreased
Decanoylcarnitine	Acetylcarnitine	Oleic acid	Phenylalanine
Hypoxanthine	Trimethyl-L-Lysine	alpha-Linolenic acid	a-Glycerophosphocholine
Uric acid	Octanoylcarnitine	Ubiquinone Q2	9-HOTrE (24h)
Xanthine		MG(18:1)	13-HODE (24h)
		MG(18:2)	TXB2 (24h)
		Palmitic acid	12-HETE (24h)
		Palmitoleic acid	11-HETE (24)
		Oxalic acid	
		Linoleic acid	
		9-HOTrE (6h)	
		12(13)-EpOME (6h)	
		9(10)-EpOME (6h)	
		9,10-DiHOME (6h)	
		12(5)-HpETE (6h)	
		LTE4 (24h)	
		LTD4 (24h)	

**Proposed biomarkers from
Untargeted and targeted approaches**

Literature on photons



UPLC-Xevo TQS

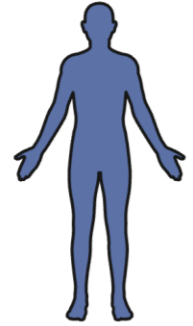
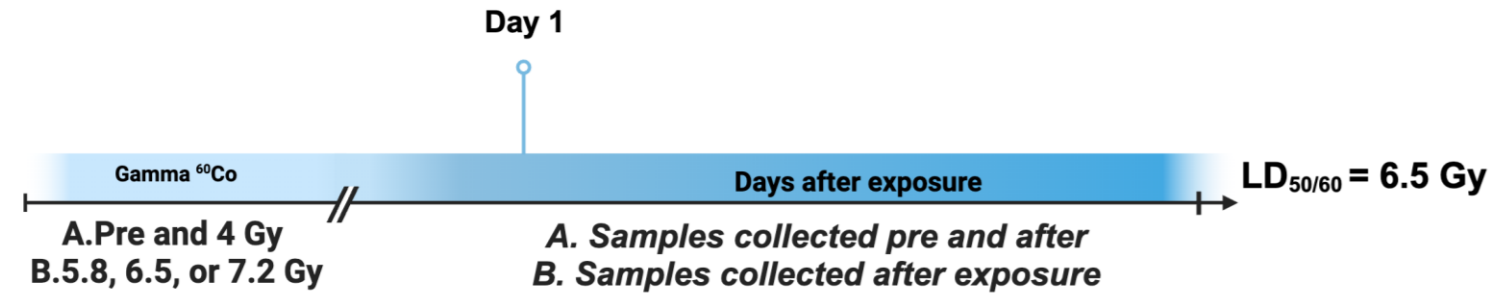




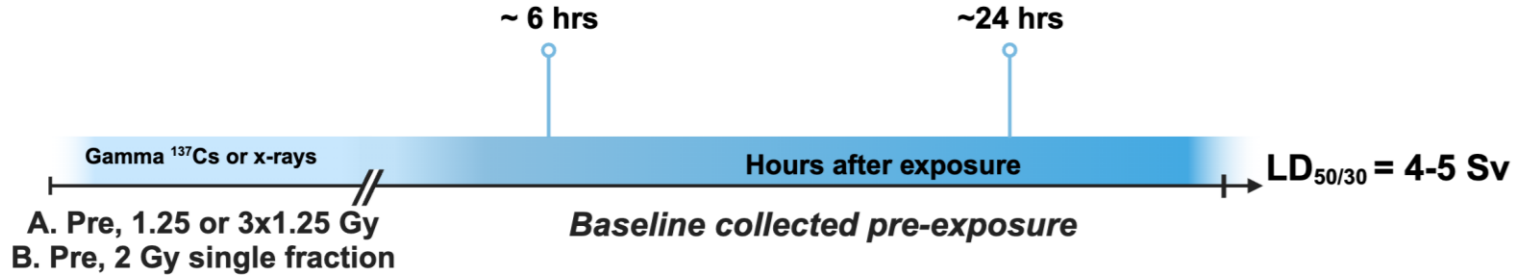
C57BL/6



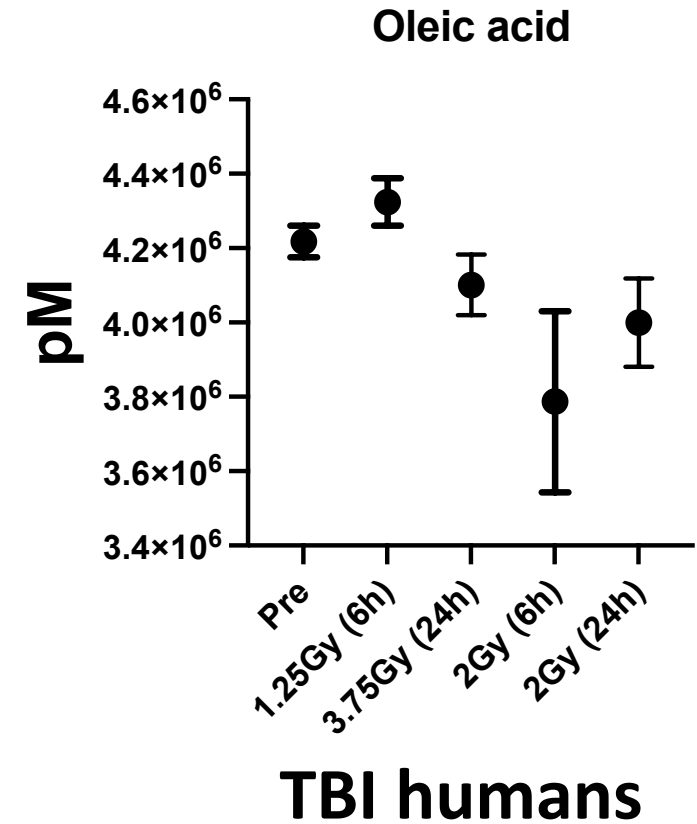
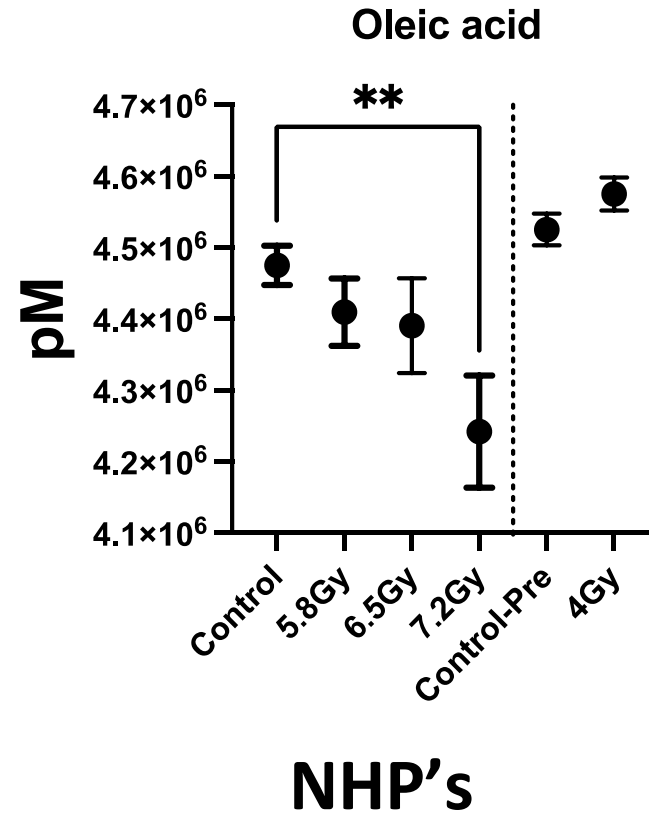
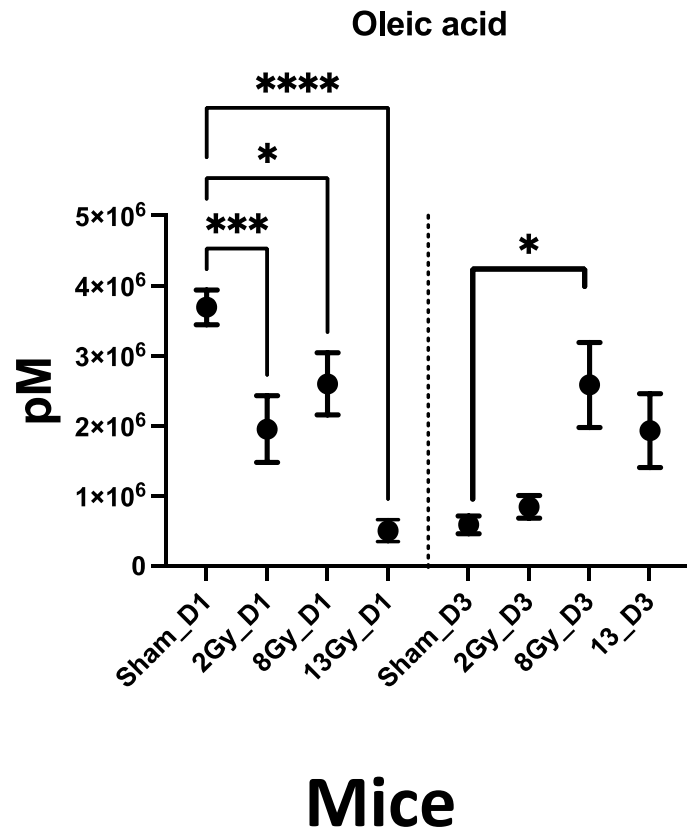
Rhesus macaques



TBI patients



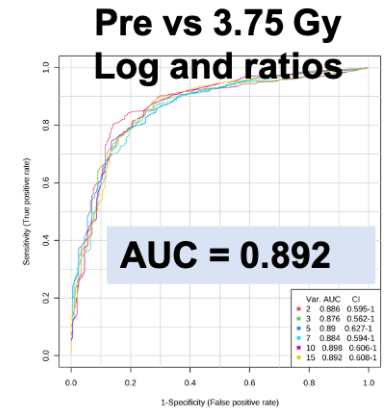
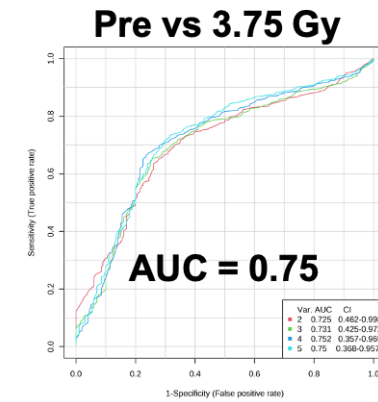
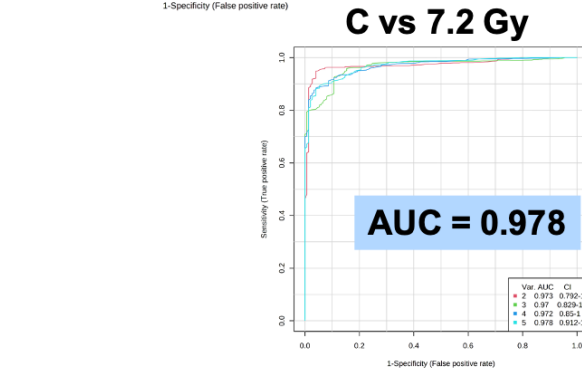
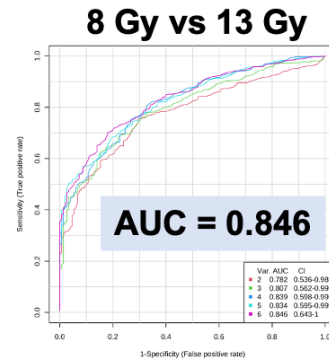
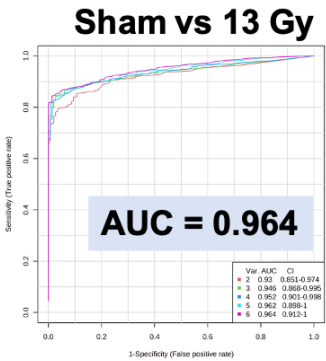
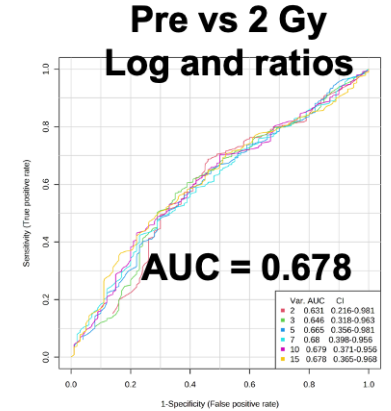
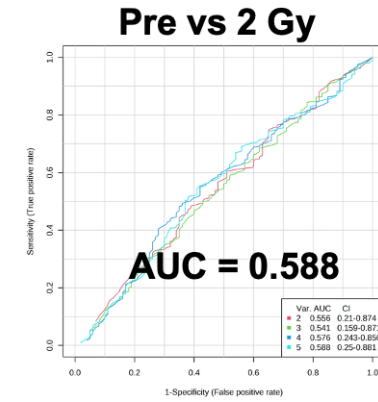
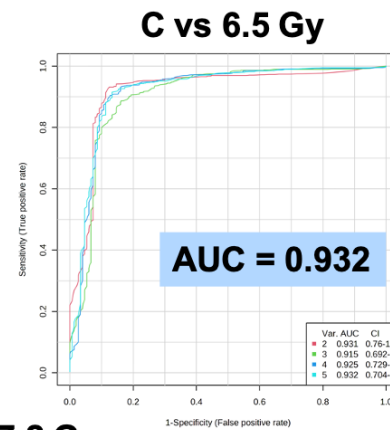
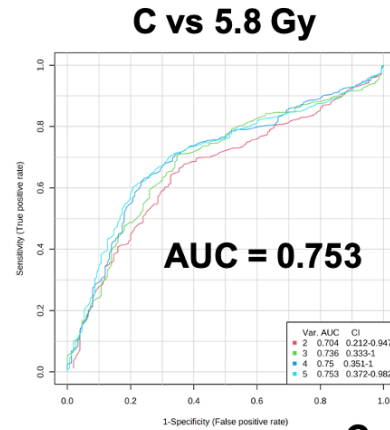
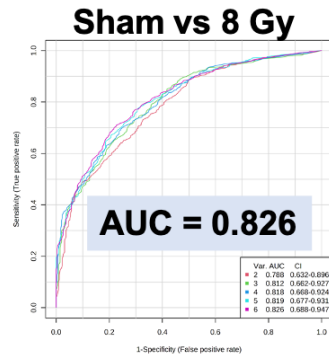
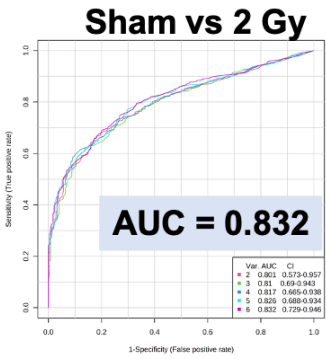
Mice – NHP's – Humans - Serum



Mice - 6 biomarker panel

NHP's - 5 biomarker panel

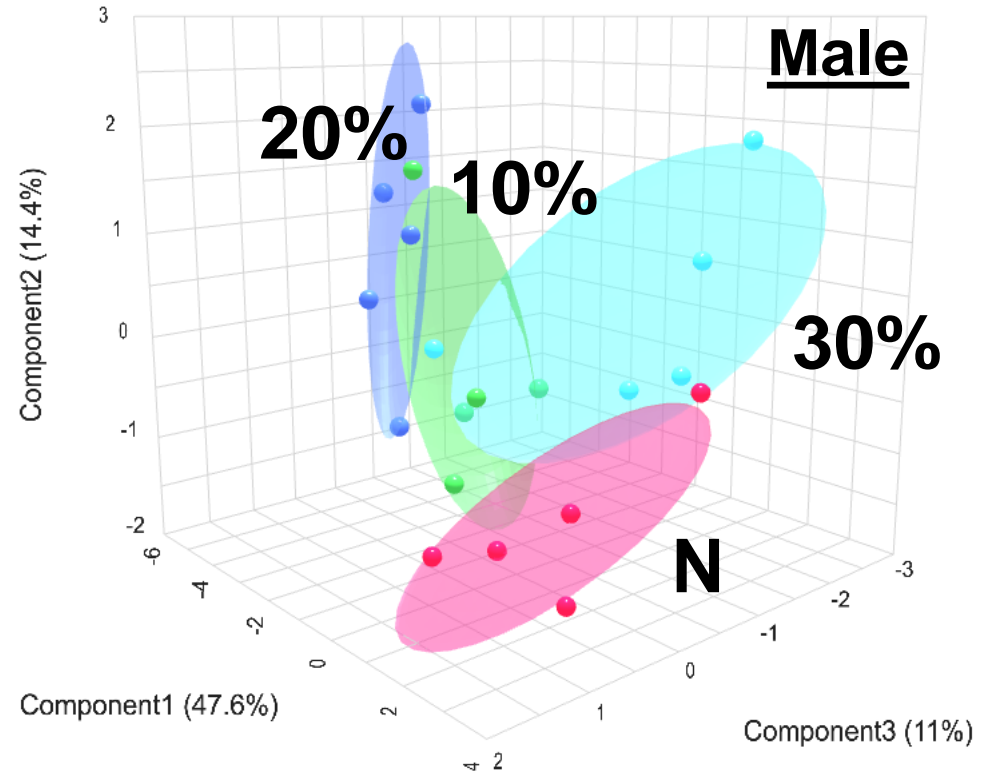
Humans - 5 biomarker panel



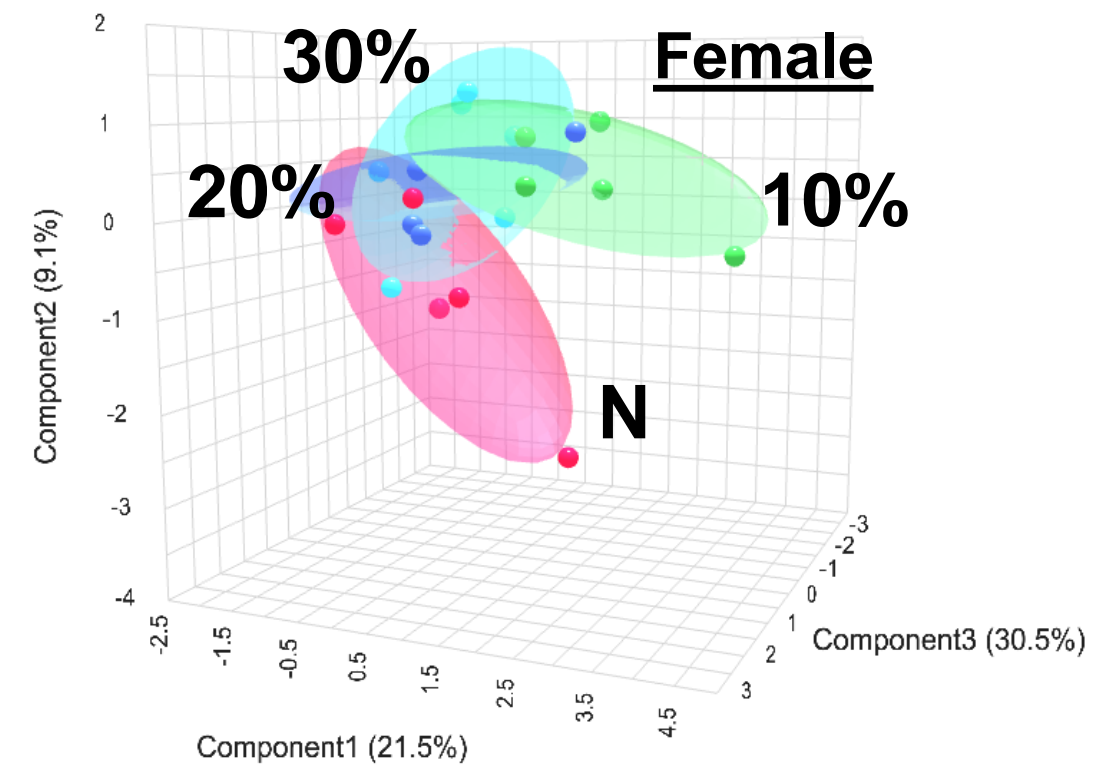
Classification by % of neutrons in total dose – serum D7

Group designation	Neutron dose	Gamma ray dose	x-ray dose	Total	Neutron fraction
C (Sham)	0	0	0	0 Gy	0%
X	0	0	3 Gy	3 Gy	0%
N	2.49Gy	0.51 Gy	0	3 Gy	83%
Mixed field				3 Gy	
10%	0.3 Gy	0.06 Gy	2.67 Gy	3 Gy	10%
20%	0.6 Gy	0.12 Gy	2.28 Gy	3 Gy	20%
30%	0.9 Gy	0.18 Gy	1.92 Gy	3 Gy	30%

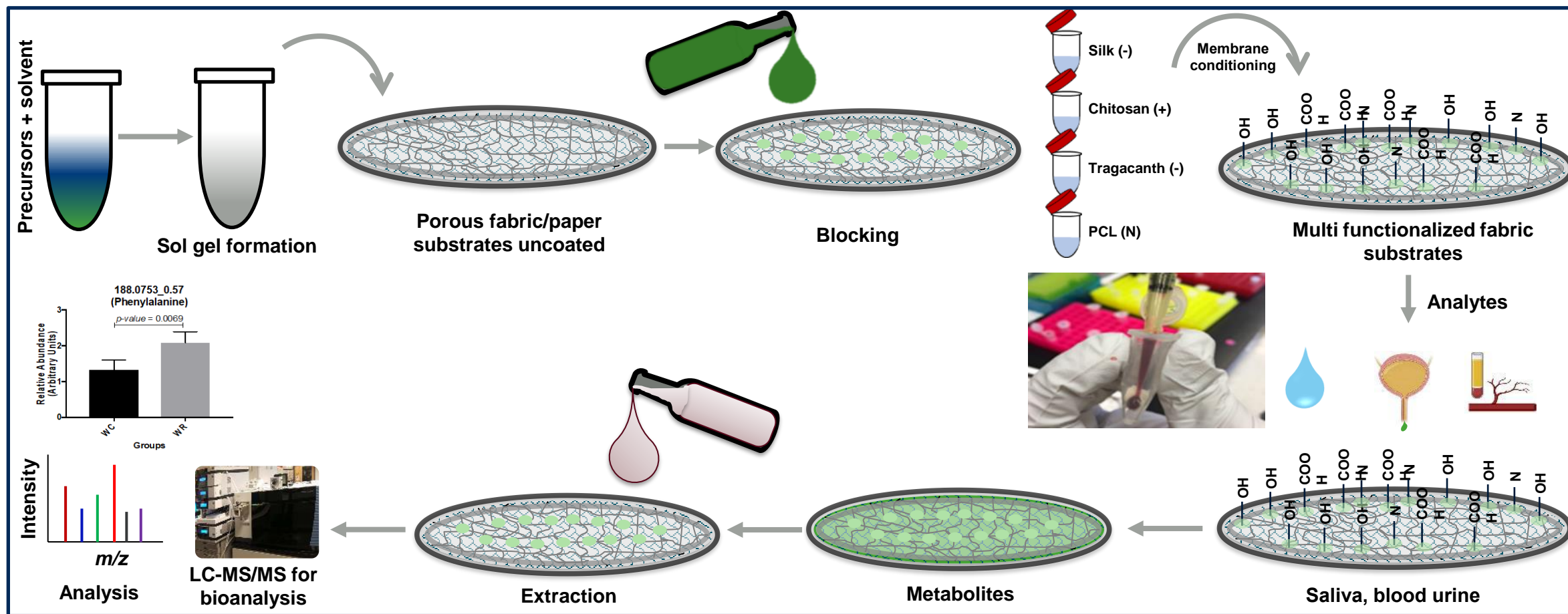
- Legend
- 1_N
 - 2_10%
 - 3_20%
 - 4_30%



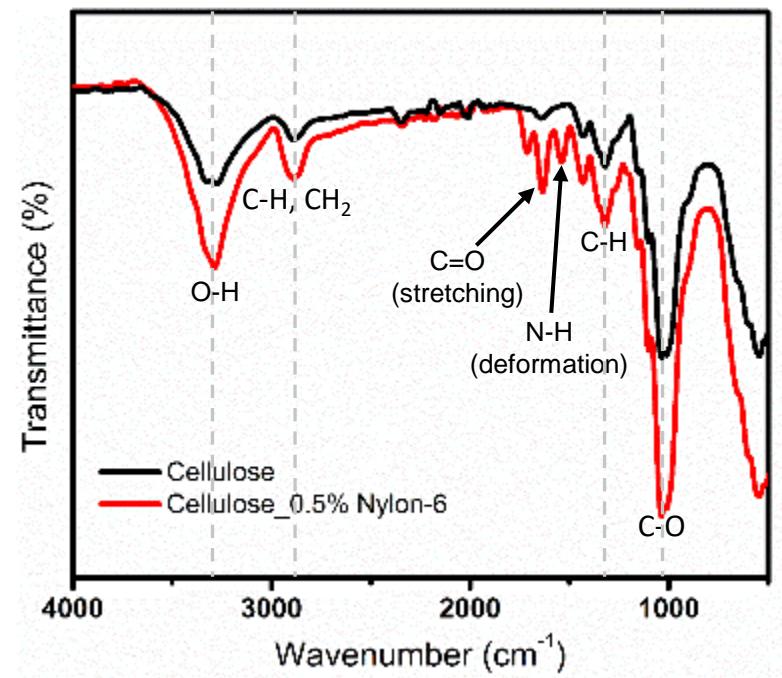
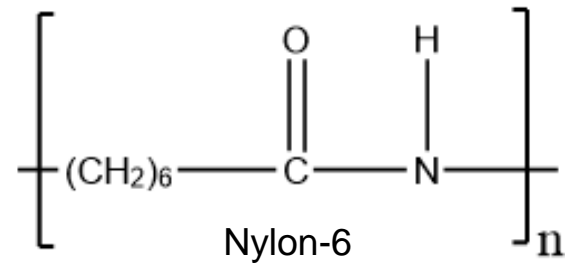
Note: Doses are physical doses



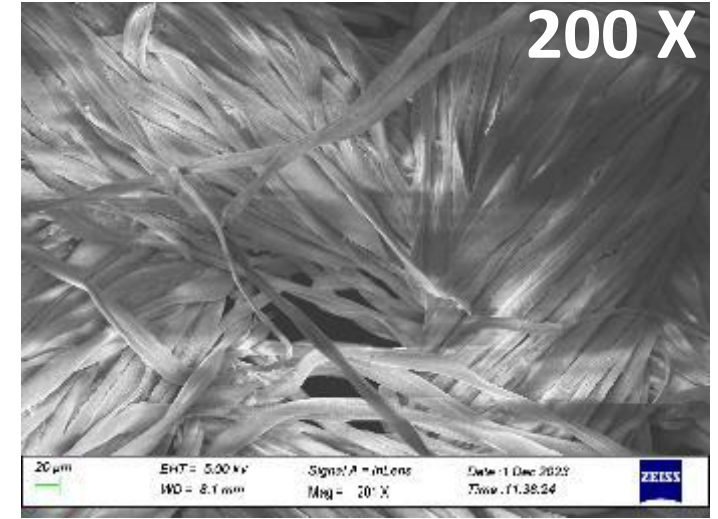
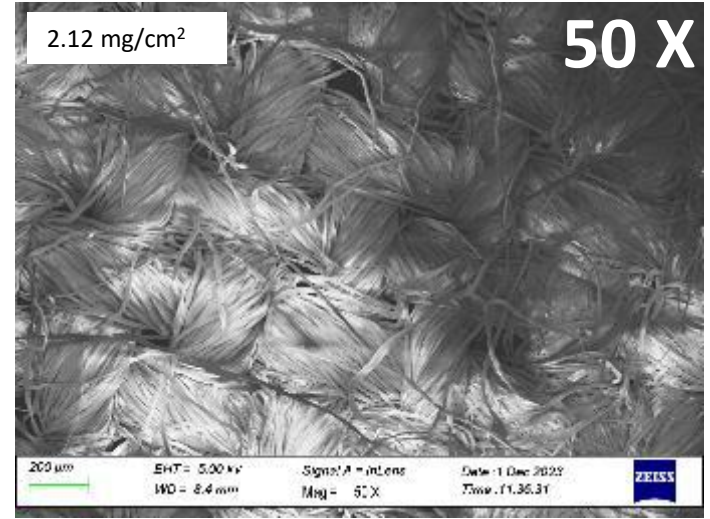
MEMBRANE SORPTIVE PHASE EXTRACTION WORKFLOW



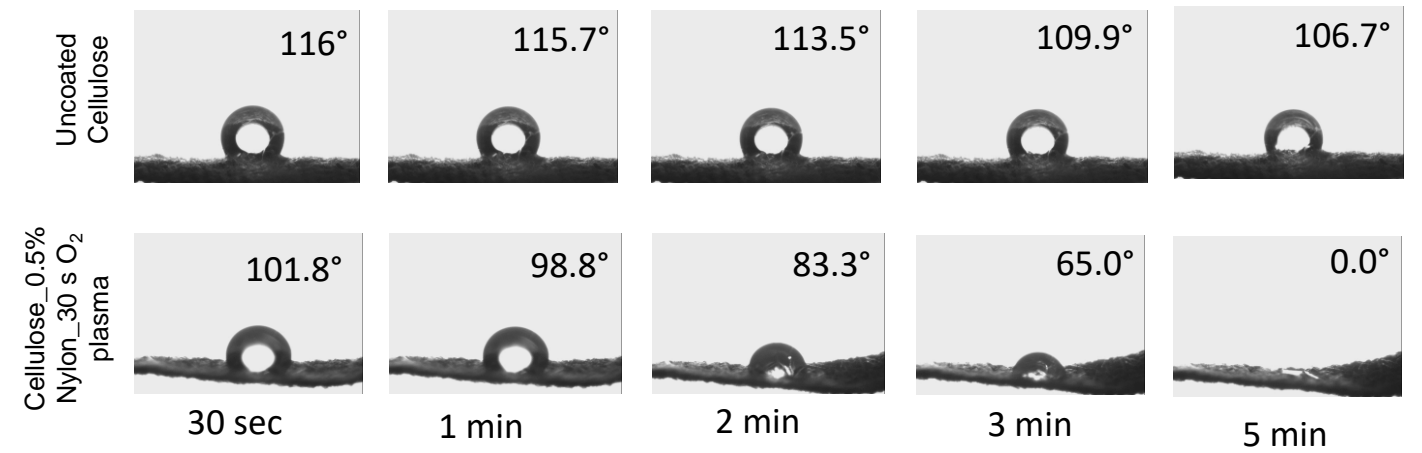
Nylon 6 Coated Cellulose Substrate



FTIR analysis of Nylon-6 coated cellulose substrate



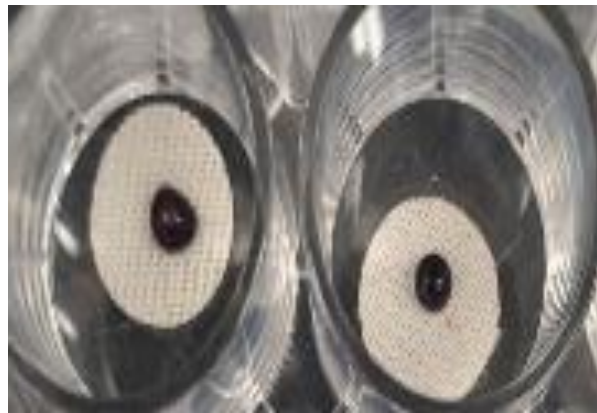
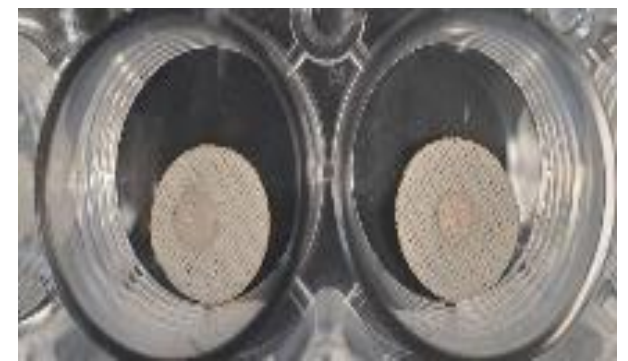
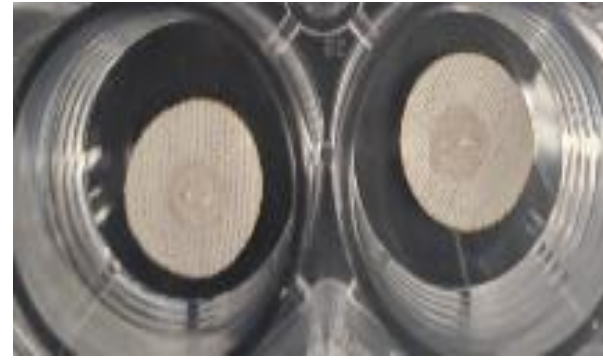
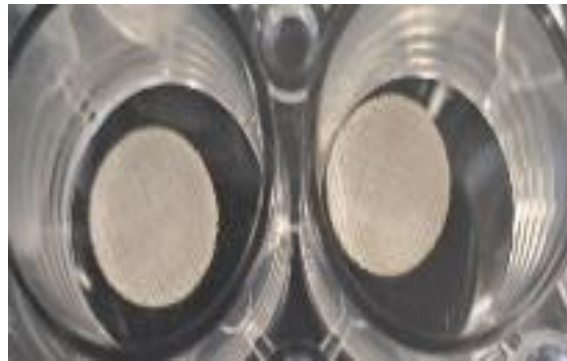
SEM images of 0.5% Nylon coated cellulose substrate

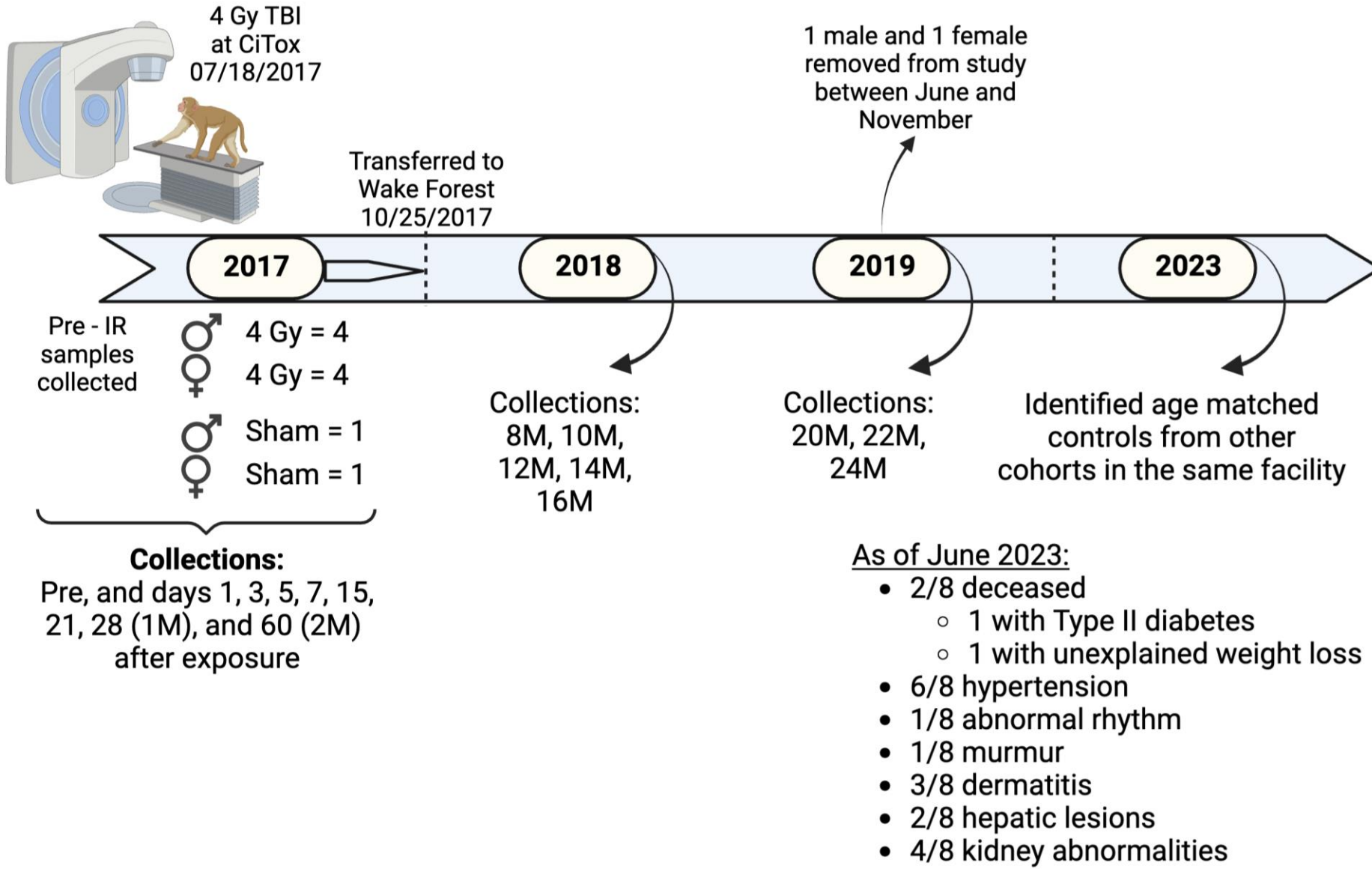


Water contact angle measurement

3391 cm⁻¹: OH, 2906 cm⁻¹: CH (stretching), 1373 cm⁻¹: CH (deformation), 1061 cm⁻¹: -C-O-
 Nylon-6 contain C=O (~1772 cm⁻¹) and N-H (~1533 cm⁻¹) groups shows in the spectra

Serum and Whole Blood Sample Analysis





20 urinary
biomarkers

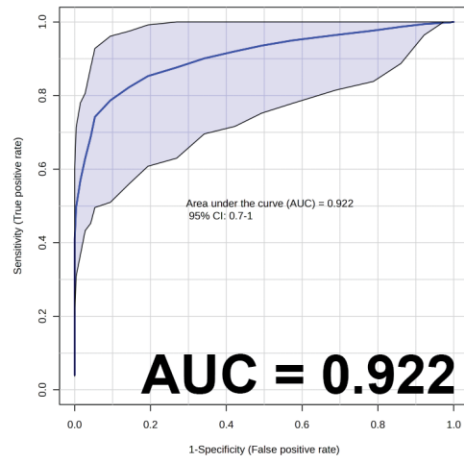
Table 1: Biomarkers for metabolomic fingerprinting

Urine	HMDB ID	m/z	ppm error	Adduct	Ret. Time	Fragment 1	Fragment 2	Fragment 3
Propionylcarnitine	HMDB0000824	218.1389	1	[M+H] ⁺	0.44	159.0663	85.0293	
Xanthurenic acid	HMDB0000881	206.0451	2	[M+H] ⁺	0.9	160.0394	132.0444	
Carnitine	HMDB0000062	144.1026	1	[M+H-H ₂ O] ⁺	0.3	103.0405	85.0297	
Uric acid	HMDB0000289	167.0209	1	[M-H] ⁻	0.3	124.0139	96.0188	
Citric acid	HMDB0000094	191.0195	1	[M-H] ⁻	0.33	111.0079		
Hypoxanthine	HMDB0000157	135.0302	8	[M-H] ⁻	0.3	92.0247	65.0139	
Taurine	HMDB0000251	124.0077	3	[M-H] ⁻	0.28	79.9565		
Pantothenic acid	HMDB0000210	218.1031	1	[M-H] ⁻	0.62	146.0811	88.0395	
Hexanoylglycine	HMDB0000701	172.0975	2	[M-H] ⁻	3.02	128.8746	99.9259	74.0241
Xanthine	HMDB0000292	151.0261	0	[M-H] ⁻	0.33	108.0195	65.9989	
Xanthosine	HMDB0000299	283.068	1	[M-H] ⁻	0.4	151.0254	108.0196	
Methylmalonic acid	HMDB0000202	117.0189	4	[M-H] ⁻	0.4	99.9256	83.9309	
Pyridoxic acid	HMDB0000017	182.0456	2	[M-H] ⁻	0.4	138.0555	108.0449	
cis-aconitic acid	HMDB0000072	173.0088	2	[M-H] ⁻	0.32	129.0171	111.0084	85.0289
Oxoglutaric acid	HMDB0000208	145.0141	1	[M-H] ⁻	0.33	101.0229	73.0312	
Glutamic acid	HMDB0000148	146.0456	2	[M-H] ⁻	0.3	128.0359	102.0543	
Malic acid	HMDB0000744	133.014	2	[M-H] ⁻	0.32	115.003		
Azelaic acid	HMDB0000784	187.0972	2	[M-H] ⁻	4.4	187.0975	125.0969	
Alanine	HMDB0000161	88.0401	3	[M-H] ⁻	0.62			
Fumaric acid	HMDB0000134	115.0034	2	[M-H] ⁻	0.33	71.0101		

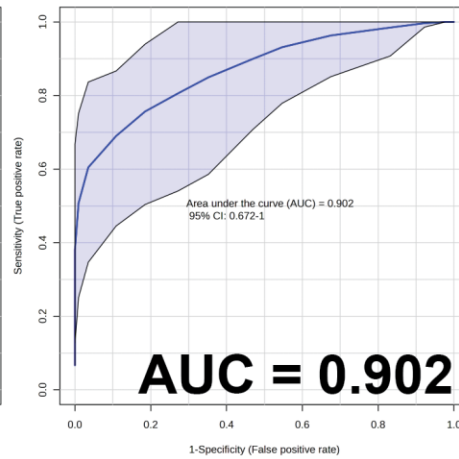
10 serum
biomarkers

Serum	HMDB ID	m/z	ppm error	Adduct	Ret. Time	mz1	mz2	mz3
Carnitine	HMDB0000062	162.1133	5	[M+H] ⁺	0.28	103.0397	85.029	
Acetylcarnitine	HMDB0000201	204.1236	3	[M+H] ⁺	0.3	145.0502	85.0291	
Eicosapentaenoic acid	HMDB0001999	325.2123	5	[M+Na] ⁺	8.2	267.2066	91.0546	
Hypoxanthine	HMDB0000157	135.0303	7	[M-H] ⁻	0.31	92.0249	65.0142	
Uric acid	HMDB0000289	167.0206	3	[M-H] ⁻	0.3	124.0204		
Citric acid	HMDB0000094	191.0188	5	[M-H] ⁻	0.44	111.0082		
Malic acid	HMDB0000744	133.0133	7	[M-H] ⁻	0.33	115.0026		
Taurine	HMDB0000251	124.007	3	[M-H] ⁻	0.28	79.9557		
Sphinganine-1-phosphate	HMDB0001383	380.2563	2	[M-H] ⁻	7.04	340.9851	315.2535	78.9588
Methylmalonic acid	HMDB0000202	117.0188	5	[M-H] ⁻	0.35	83.9311		

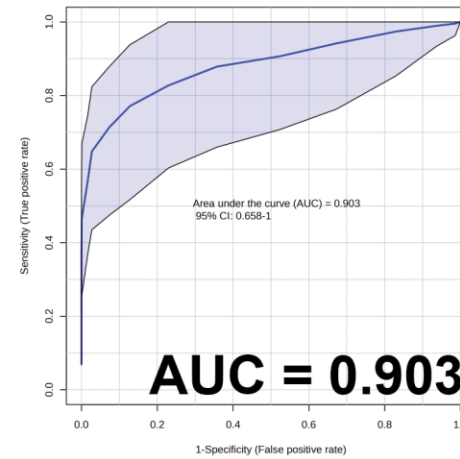
Urine - 20 biomarkers



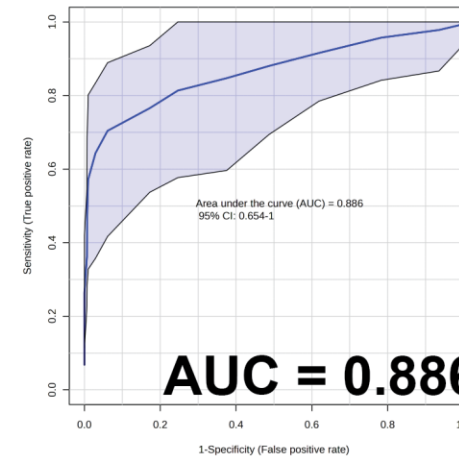
AUC = 0.922



AUC = 0.902

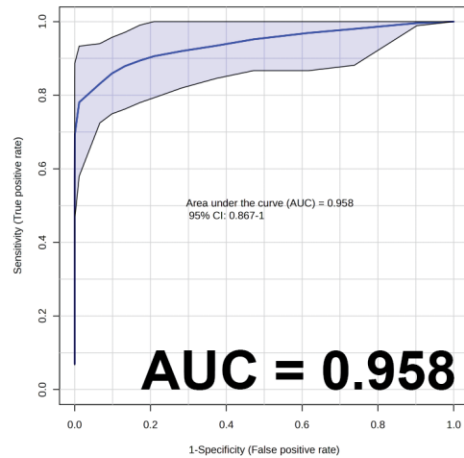


AUC = 0.903

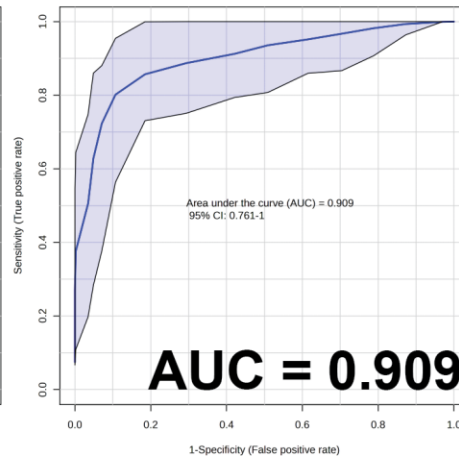


AUC = 0.886

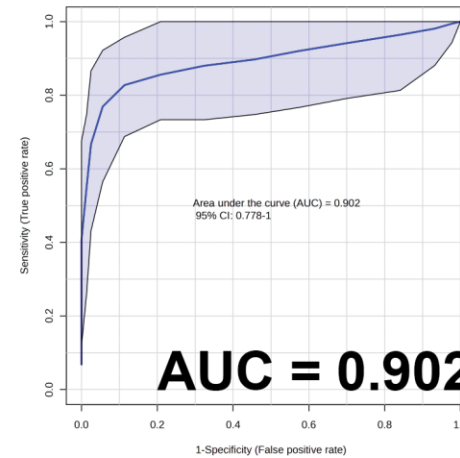
Serum - 10 biomarkers



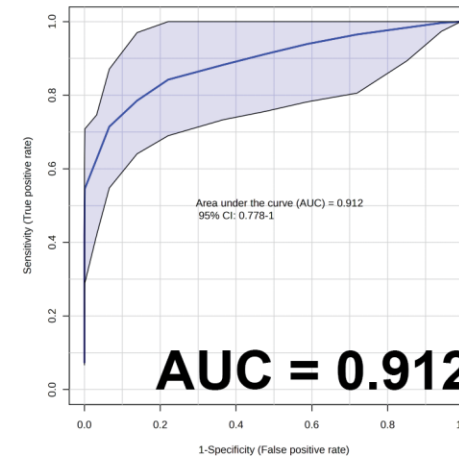
AUC = 0.958



AUC = 0.909



AUC = 0.902



AUC = 0.912

Pre vs D1

Pre vs D7

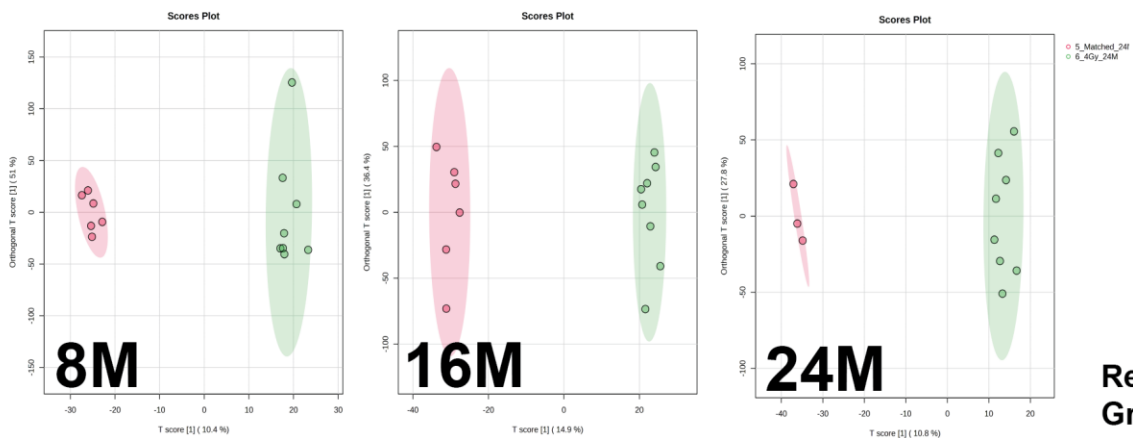
Pre vs 1 month

Pre vs 2 months

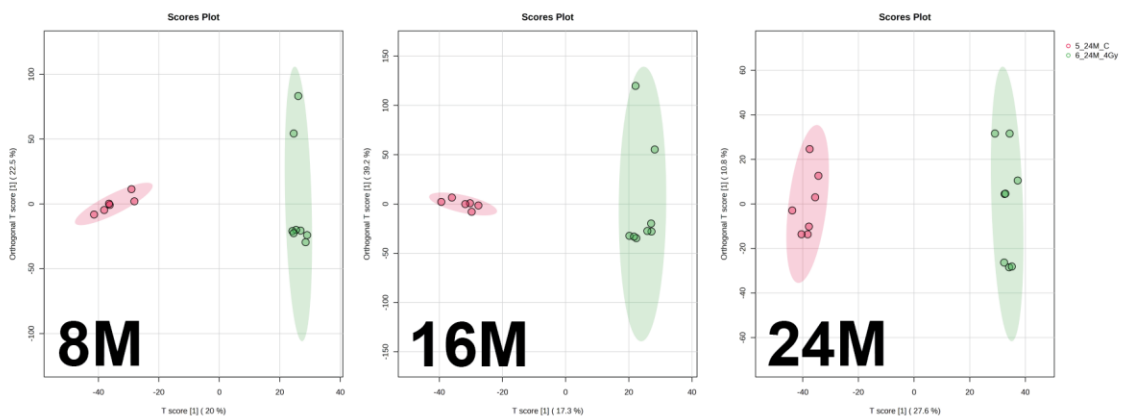
But what about age? Used age matched controls

A.

URINE

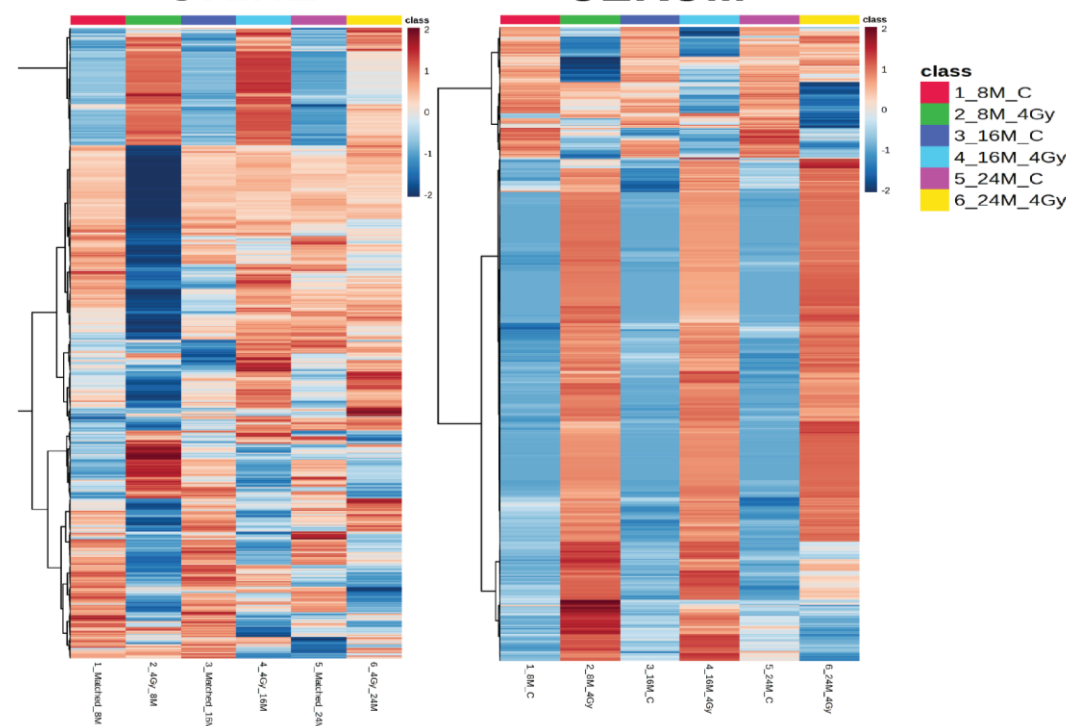


SERUM



Red: Controls
Green: 4 Gy

URINE



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