Florian FORMANEK, PhD

Global Life Sciences Market Manager

HORIBA Scientific

Fluorescence Fingerprinting for Biotherapeutics Characterization

EPIC

EUROPEAN PHOTONICS INDUSTRY CONSORTIUM

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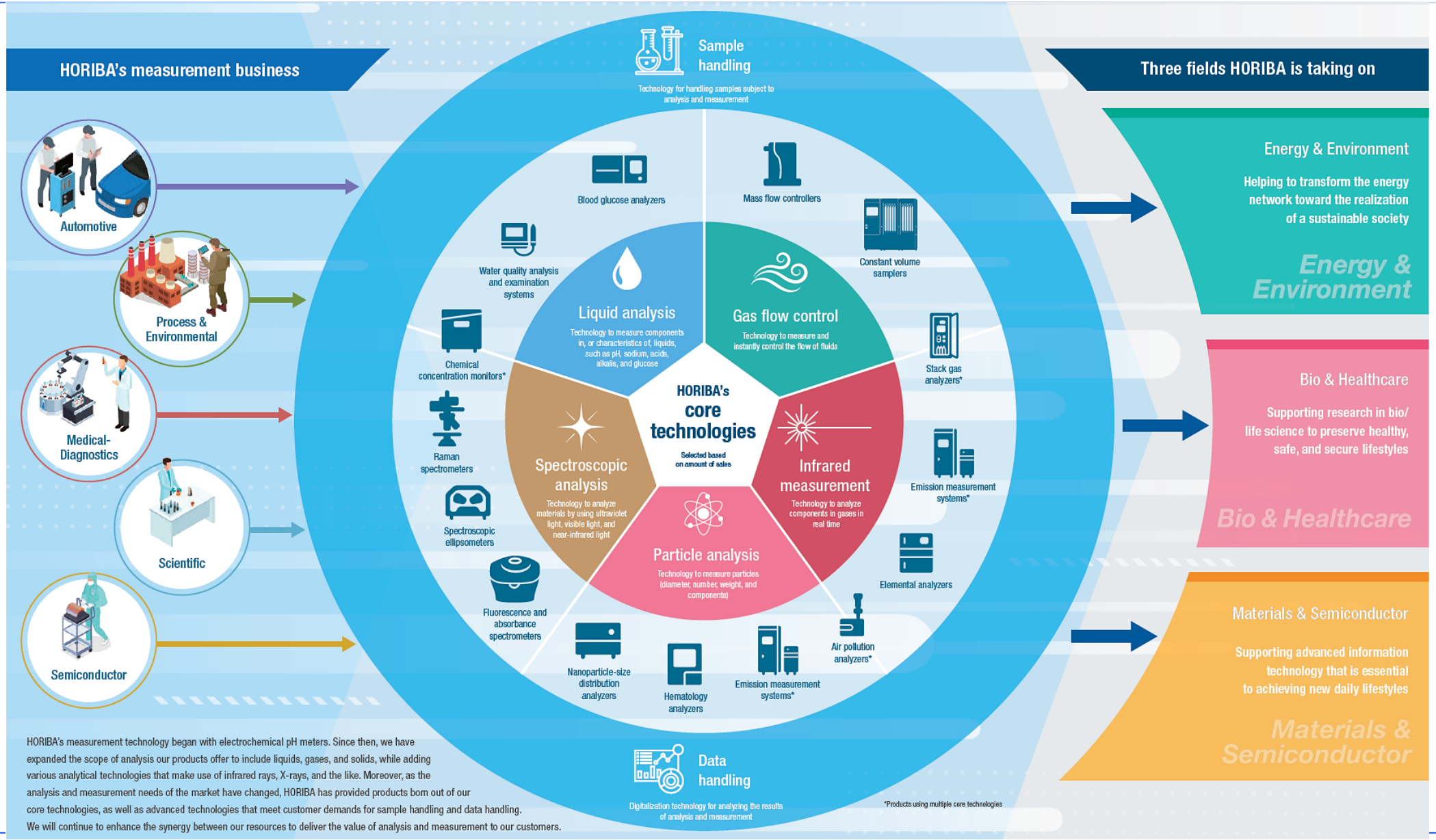
HORIBA's measurement technology



HORIBA's measurement technology began with electrochemical pH meters. Since then, we have expanded the scope of analysis our products offer to include liquids, gases, and solids, while adding various analytical technologies that make use of infrared rays, X-rays, and the like. Moreover, as the analysis and measurement needs of the market have changed, HORIBA has provided products born out of our core technologies, as well as advanced technologies that meet customer demands for sample handling and data handling. We will continue to enhance the synergy between our resources to deliver the value of analysis and measurement to our customers.

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What spectroscopy brings to Pharma

Spectroscopy

- Very fast
- Low Carbon foot-print
- Low cost

HPLC

- No matrix effect
- Sensitive

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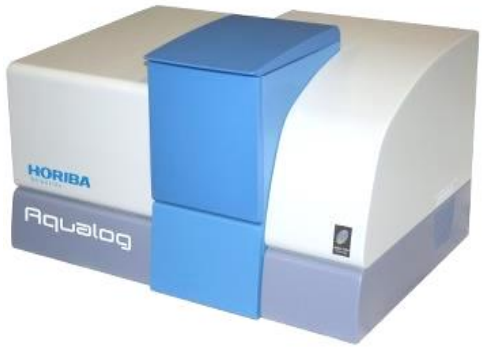
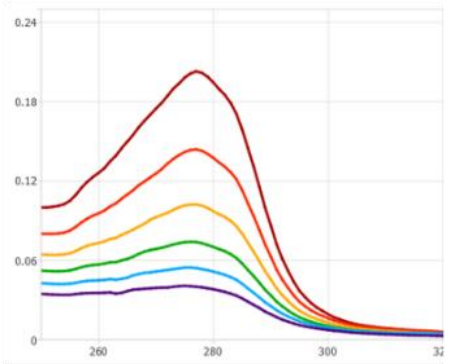
A-TEEM* – Clear molecular fingerprint

A-TEEM uses the simultaneous measurement of Fluorescence and Absorbance to detect and quantify components in complex matrices.

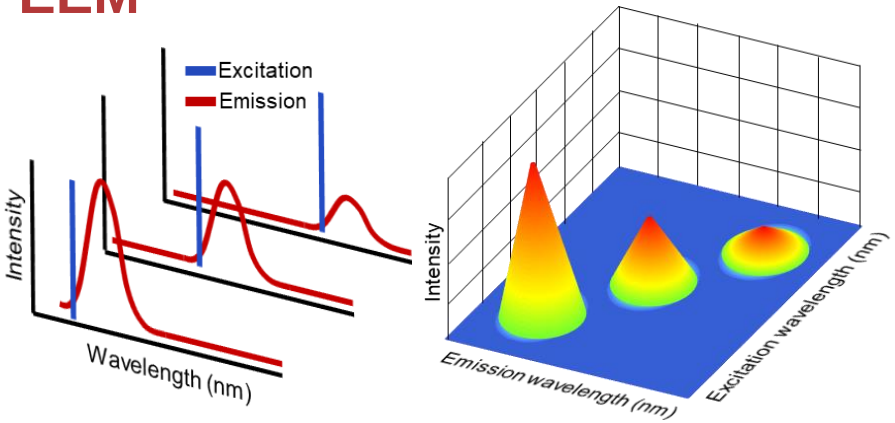


Data Collection: Same Sample @ Same Time

Absorbance



EEM



***Absorbance – Transmission Excitation Emission Matrix**

Analysis in seconds
- 30-60 sec data acquisition

Simple sample prep
- Liquid samples only
- Extract with small amount of solvent

Cost effective
- No columns, solvents or waste disposal fees

Molecular ID of unknowns
- Chromatography can't touch this

Low LODs
- Orders of magnitude better than Raman
- PPM→PPB even in complex matrices

High sensitivity and selectivity

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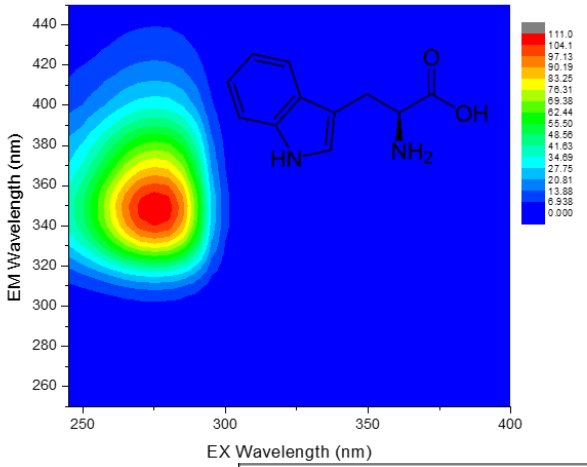
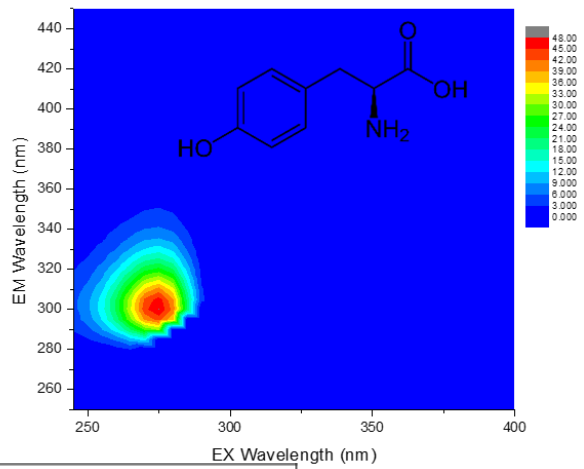
EEM – Selective, low concentrations

Protein Signatures
Vary with Environment

Tryptophan

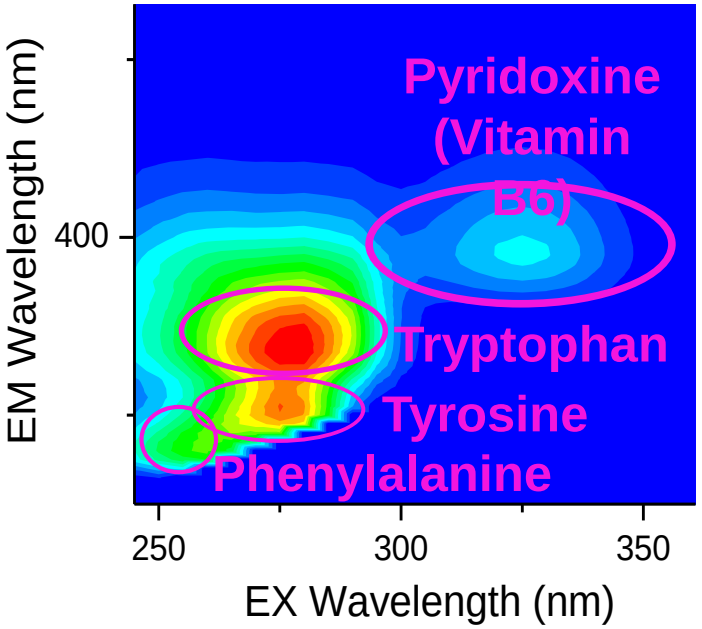


Tyrosine



Literature Values [G oza, 2016]			
Amino Acid	**λex (nm)**	**λem (nm)**	**Comments**
Tyr (Y)	275	305	
Trp (W)	275	330-332	Non-Polar
		340-342	Limited-polar
		350-353	Polar-exposed

Complex Samples
Distinct Patterns



Low Concentrations ⟶ mg/mL

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A-TEEM insensitive to interering compounds

Many components that contribute to complex matrices for vibrational spectroscopy (water, sugar, etc.) are invisible to A-TEEM

	A-TEEM	Raman	FTIR/NIR
Simple Sugars	N/A	🔴	🟡
-OH; -NH; -CH	N/A	🟡	🟡
Water	N/A	🟡	🔴

Invisible to A-TEEM

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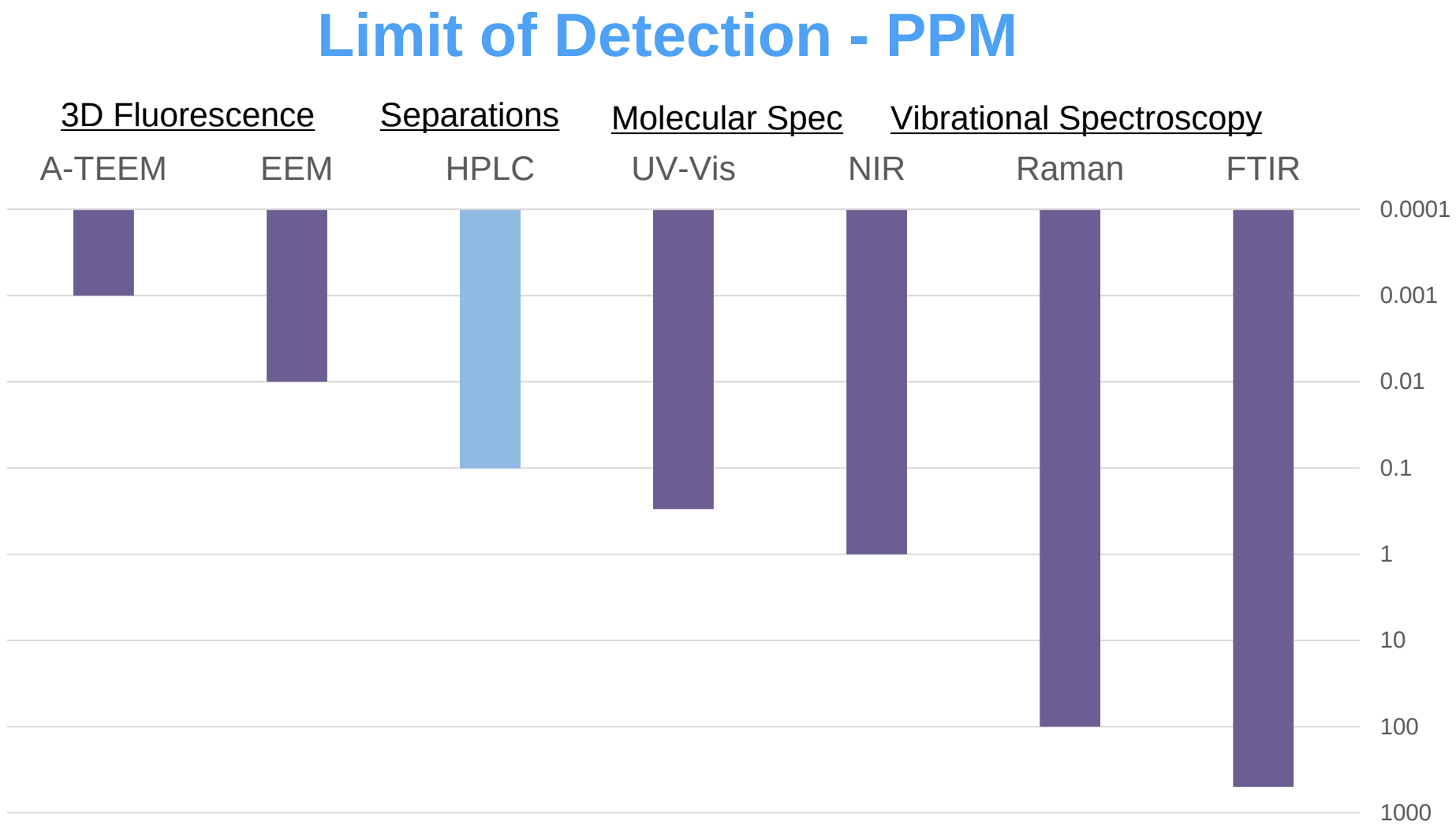
Quantitative analysis – low concentrations



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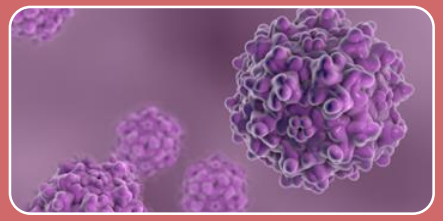
Applications in biopharma

Vaccines

- Differentiate similar multicomponent vaccines with 100% accuracy
- Identify post-translational mod's (glycosylation or sulfonation)
- Differentiate old vs new batch (aggregation) / Reveal amino acid substitutions

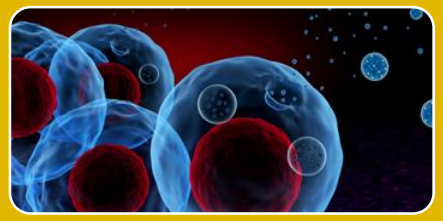
Cell Culture Media

- Differentiate similar/related media
- Observe degradation under storage conditions



AAVs

- Resolve AAV2 from AAV9 serotypes (surface protein characterization)
- Accurately quantify the payload filling percent



Exosomes

- Differentiate exosomes populations

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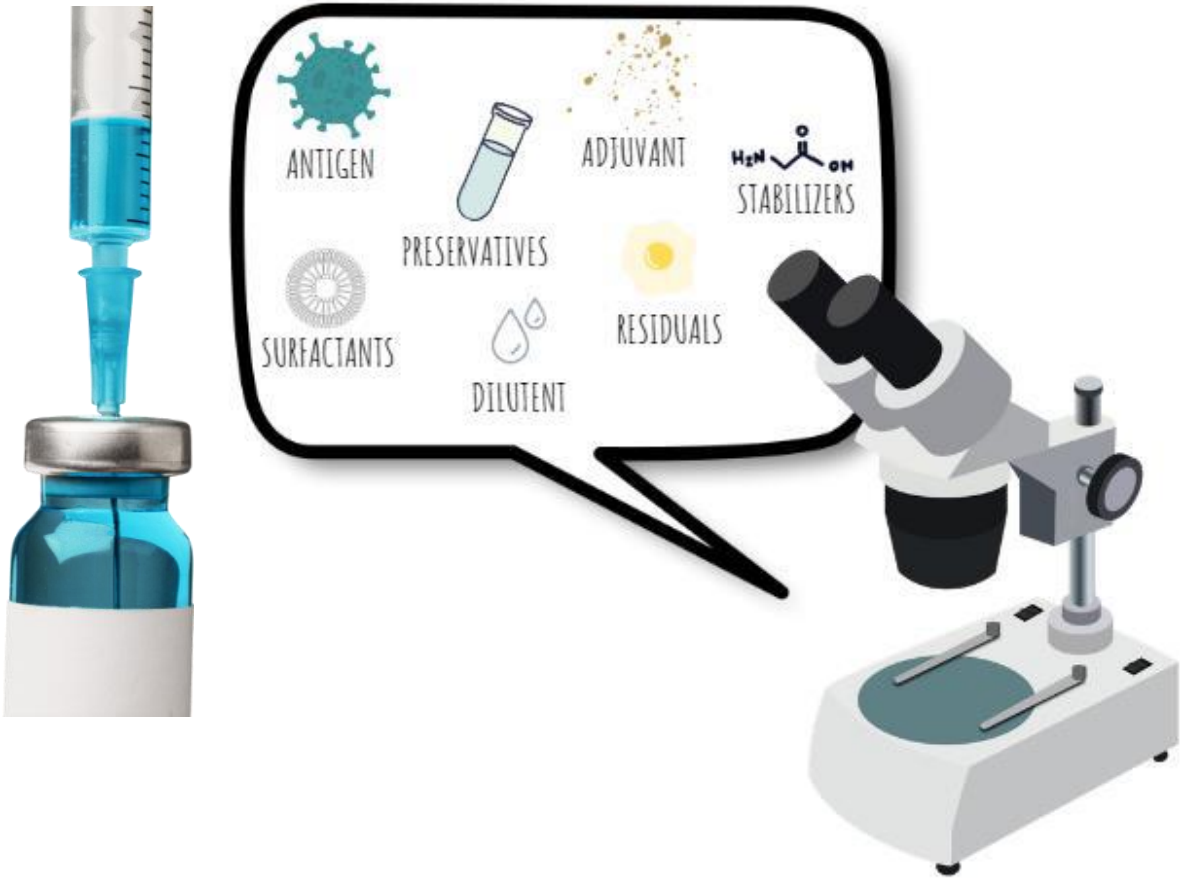
Vaccines – Vibrational spectroscopy ?

Vibration Spectroscopy – go-to option for **rapid analysis**

- Struggle with vaccine samples



No problem for fluorescence

Shingrix - lyophilized powder	Amount	Role	Concentration
Glycoprotein E			0.1 mg/ml
Sucrose			40 mg/ml
Polysorbate 80	0.8 mg	Excipient	
Sodium Dihydrogen Phosphate	0.16 mg	Excipient	
Dipotassium phosphate	0.116 mg	Excipient	
Water-based diluent			

Challenge for Raman

Challenge for FTIR/NIR

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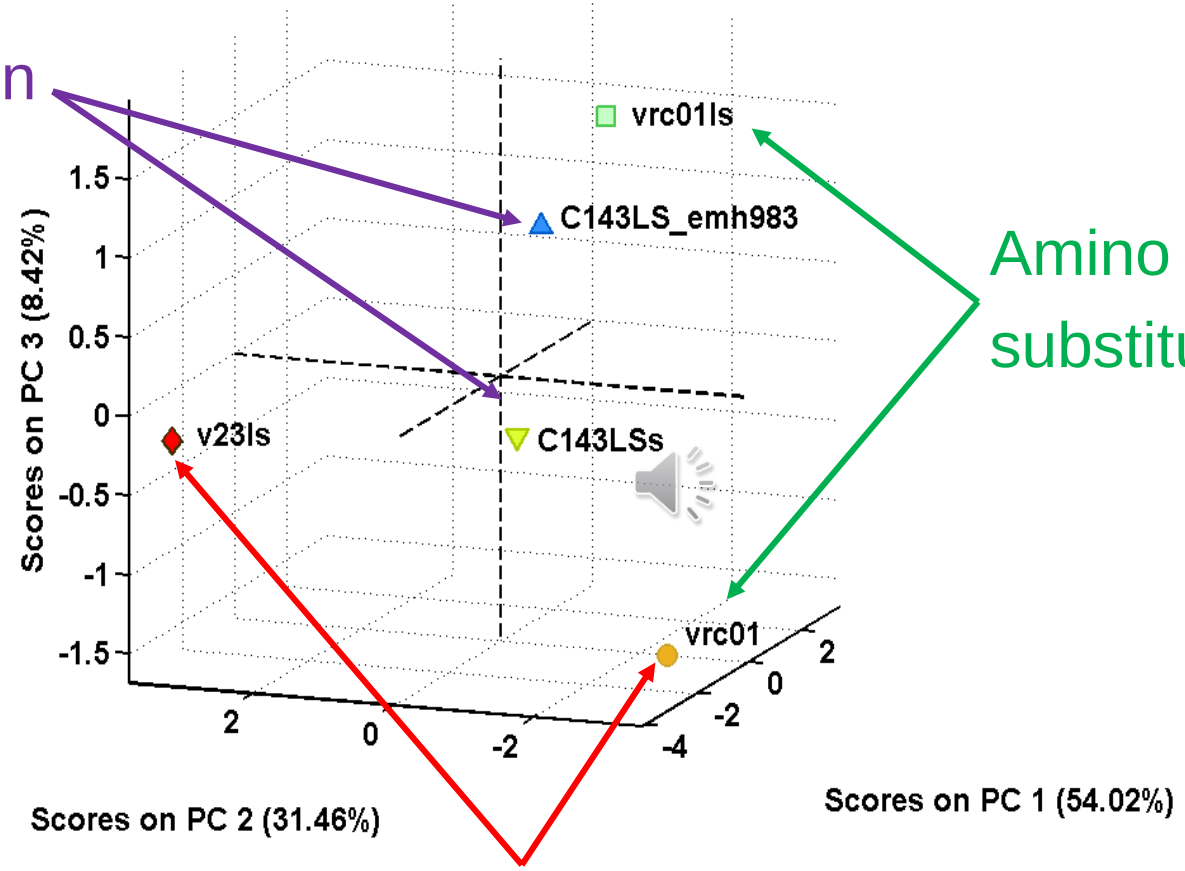
Vaccines differentiation

Aggregation



Amino acid substitution

Estimate LoD
0.15 µg/mL

Post-translational modification

Vaccines provided by the Vaccine Research Center

NIH National Institute of Allergy and Infectious Diseases

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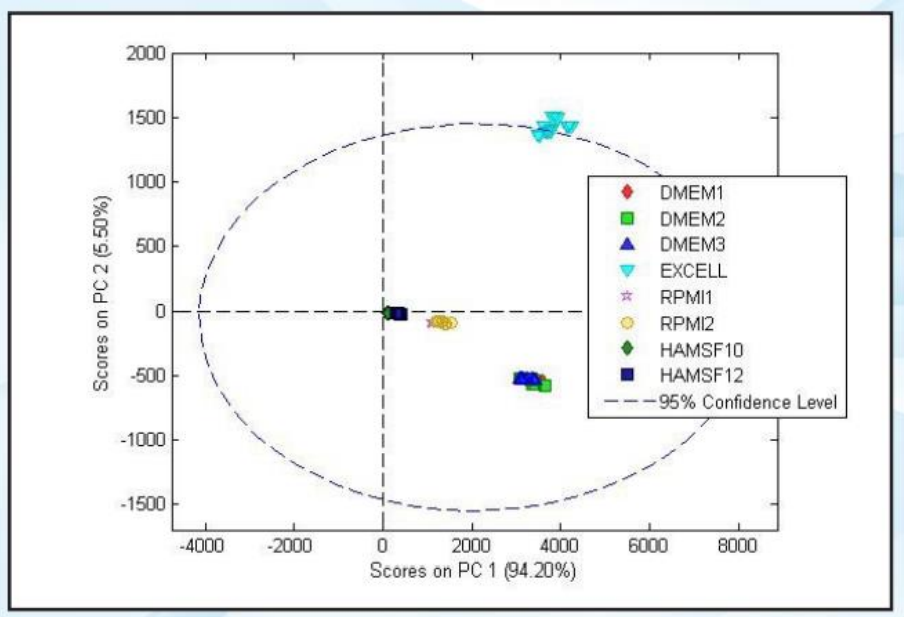
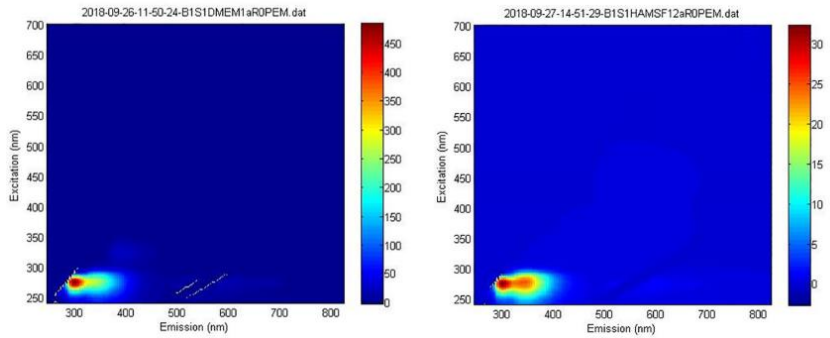
Cell media – classification & quality check

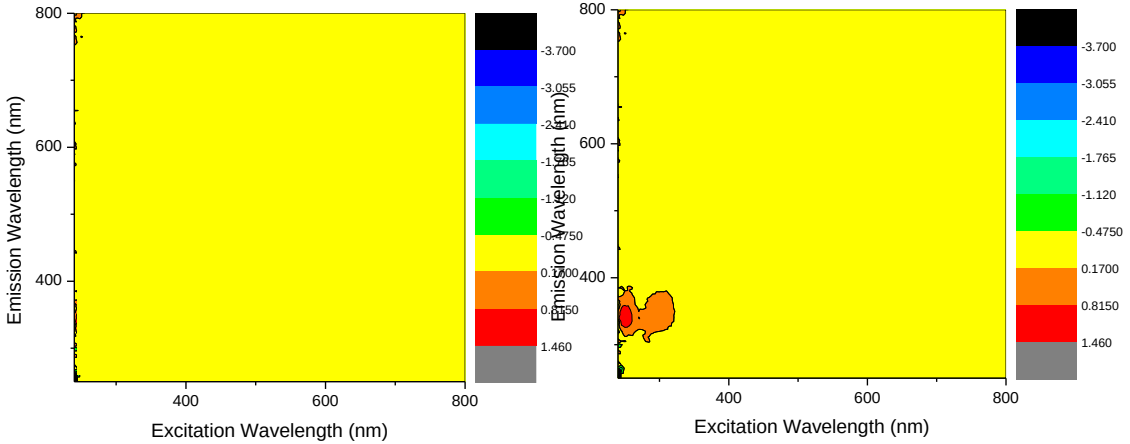




Differentiate

No features in A-TEEM:
Unknown = “GOOD”

Feature in A-TEEM:
Unknown = “Out of Spec”



Subtract UNKNOWN From GOOD

Compare

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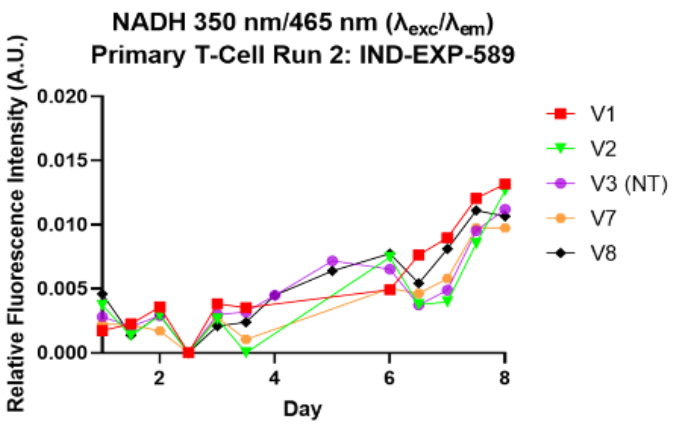
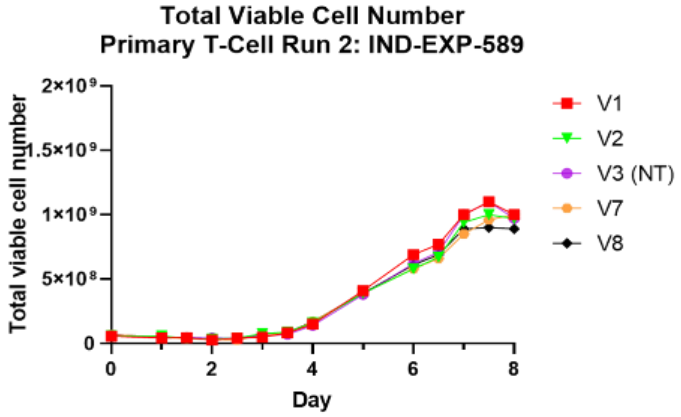
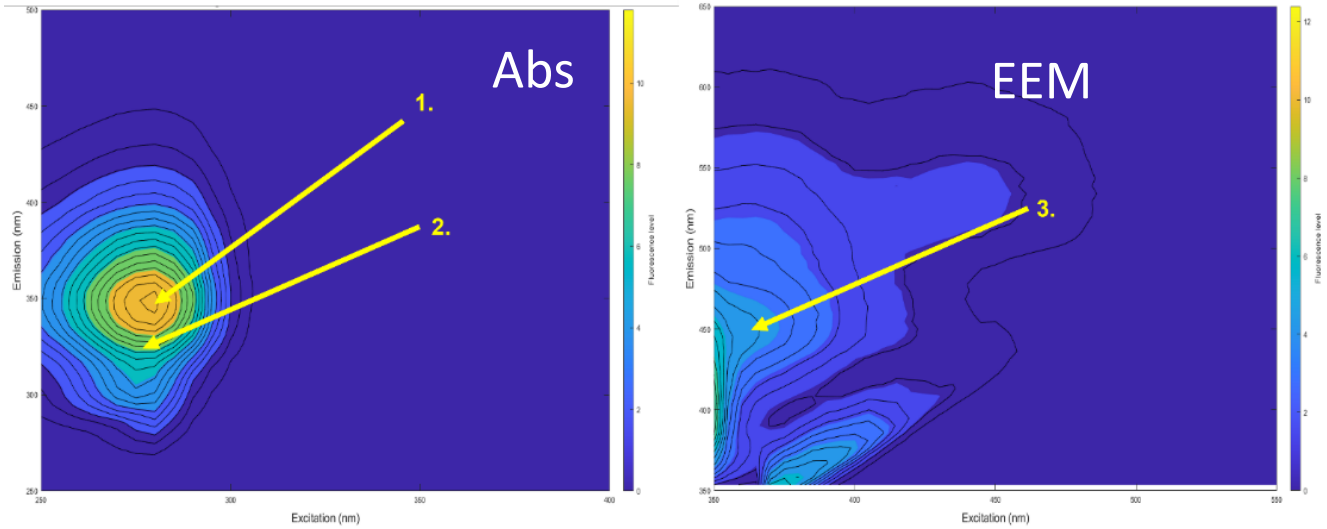
Bioprocess monitoring – cell therapy







Partnership with the Cell and Gene Therapy Catapult as part of the Process Analytical Technology Consortium

- Spectral data requires pre-processing
- EEM regions of fluorescent species:
  - **1. Tyrosine**
  - **2. Tryptophan**
  - **3. NAD(P)H**
- NAD(P)H can be related to cell proliferation → trend comparison to total viable cell shows correlation

CATAPULT
Cell and Gene Therapy

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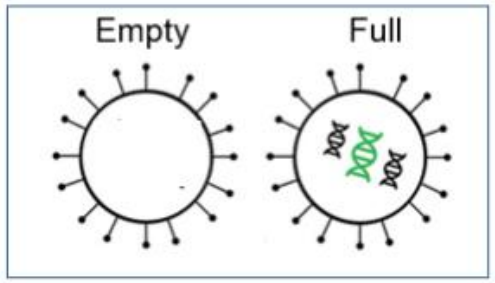
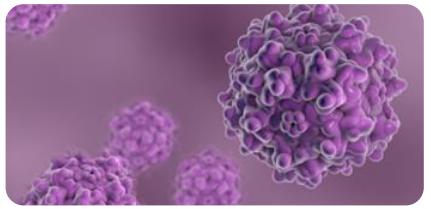
AAVs for targeted delivery



Empty/Full ratio is Important Manufacturing Quality Attribute



Serotype	Part Number	Physical Particle Count / mL	% Empty	Empty or Full
AAV2	RS-AAV2-ET	1.27×10^{12}	99.5%	Empty
AAV9	RS-AAV9-ET	1.76×10^{12}	93.1%	

Serotype	Part Number	Vector Genome Count / mL	% Full	Empty or Full
AAV2	RS-AAV2-FL	1.82×10^{11}	71.2%	Full
AAV9	RS-AAV9-FL	3.86×10^{11}	82.3%	

AAV = Adeno-Associated Viruses

Serotype Targets	
	
Kidney Eye Brain Liver Joint Lung Muscle	Liver Muscle Lung

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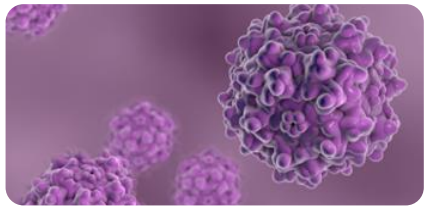
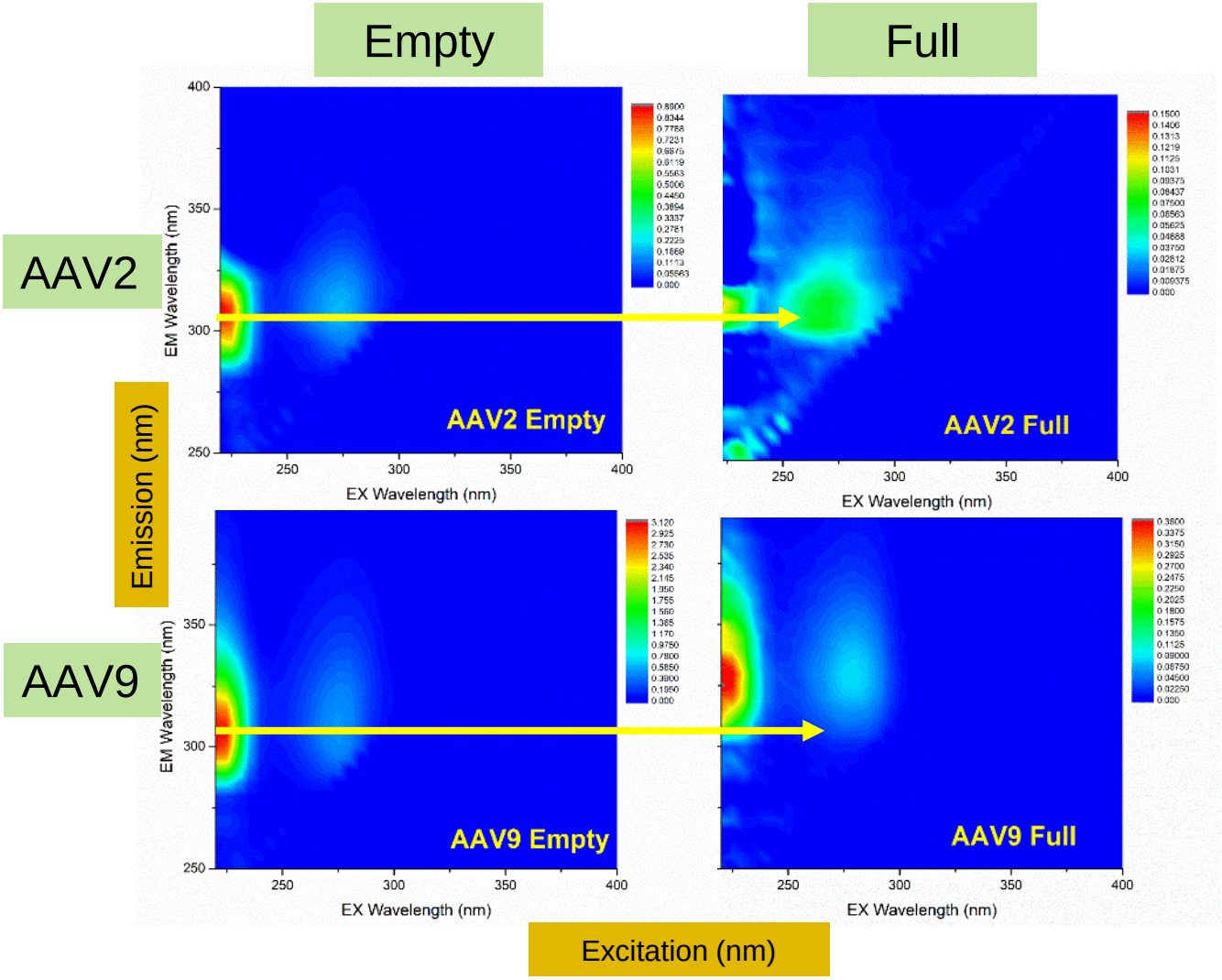
AAVs capsids





- Em. Max shifts for filled AAV2 & AAV9 capsids
- Full capsids are less fluorescent than empty ones

Rapid screening:

- **Resolving serotypes**
- **Empty / full capsids**

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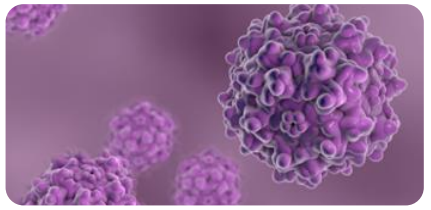
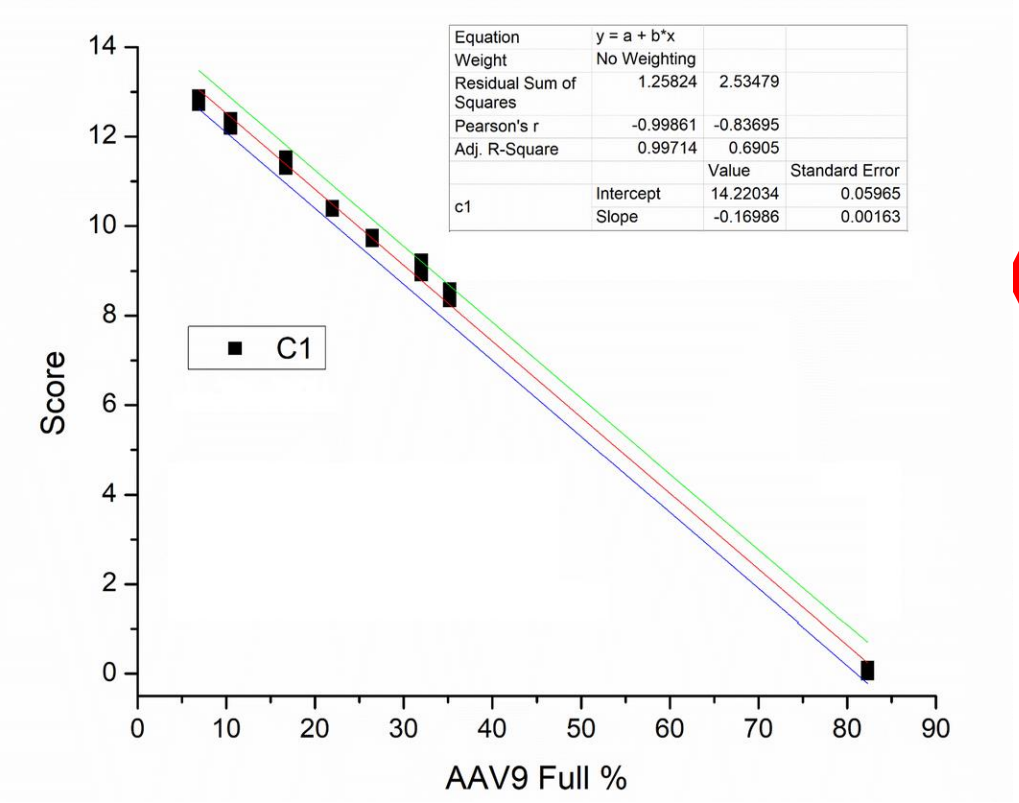
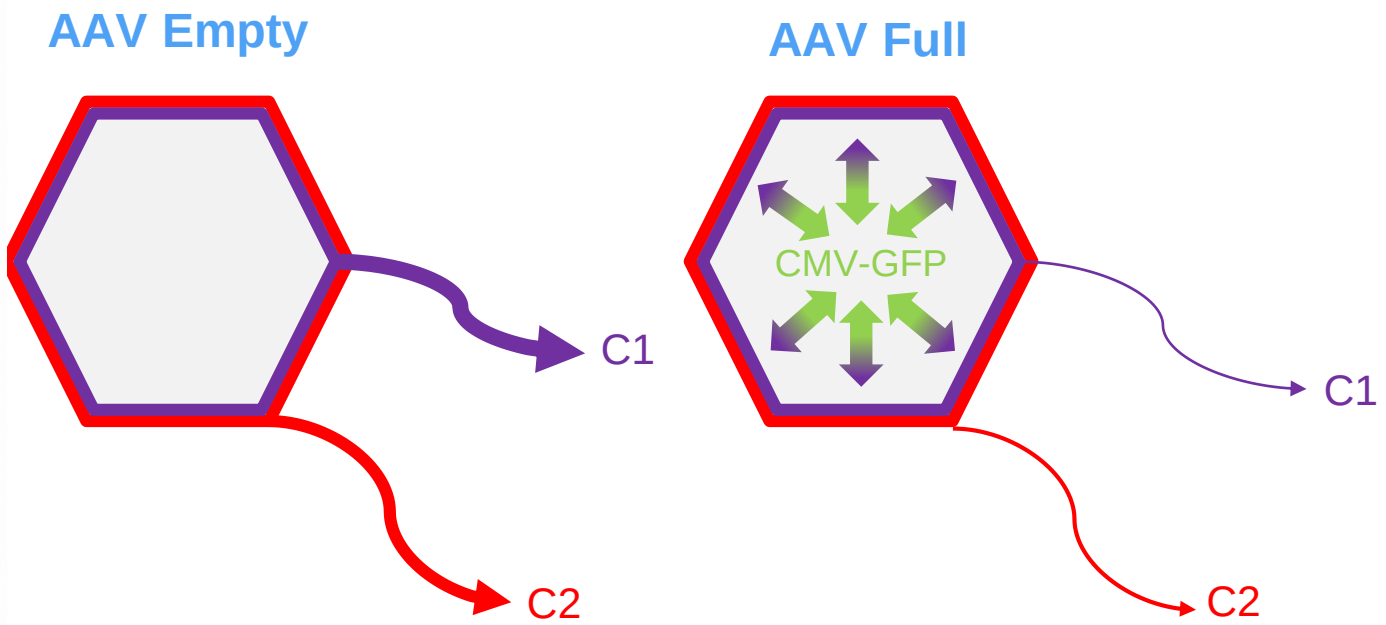
AAVs loadings ratio…



- Parafac component scores plotted against “full” metric provided by AAV manufacturer
- Component 1 (Tyr) fluorescence is quenched (linearly) by the DNA





- Component 1 (Tyr) fluorescence is quenched (linearly) by the DNA payload

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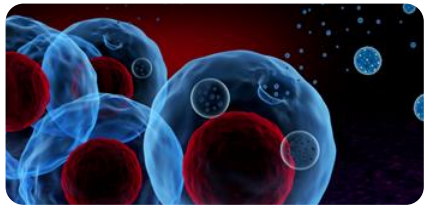
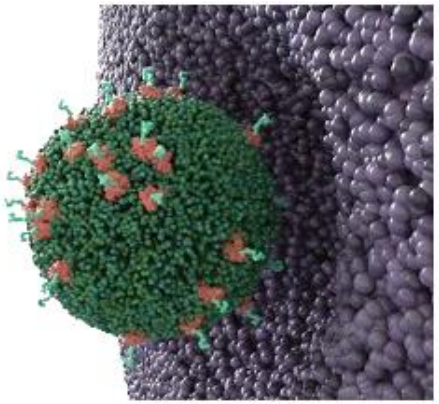
Exosomes for drug delivery

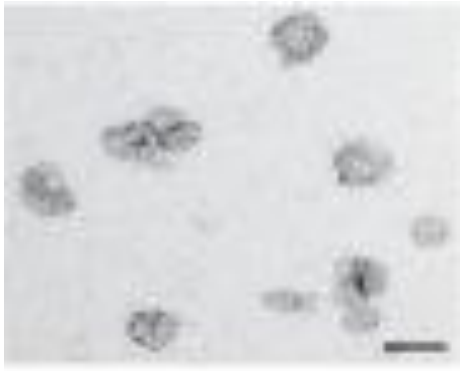




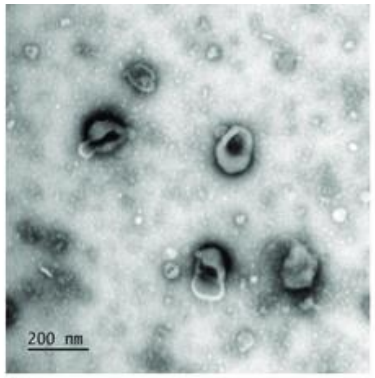
Credit: Evox Therapeutics

Exosomes can deliver therapies into cells that would otherwise be hard to reach.

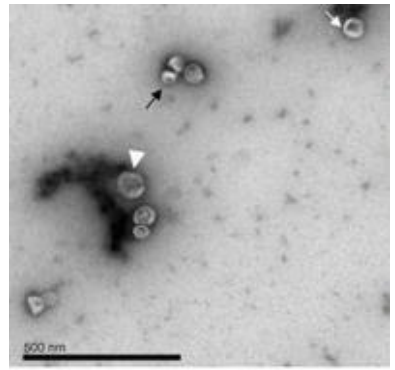
Company	Location	Progress		
		Discovery	Preclinical	Phase 1
Codiak BioSciences	America	5	2	2
The Cell Factory	Belgium	2	2	0
United Therapeutics	America	0	0	1
Avalon GloboCare	America	0	2	0
Unicyte AG	Germany	0	1	0
Tavec Pharmaceuticals	Canada	0	1	0
Evox Therapeutics	Britain	1	0	0



PC3 Exosomes derived from prostate cancer cell line



MCF7 Exosomes derived from human breast cancer cell line



HEK 293 Exosomes derived from human embryonic kidney cells

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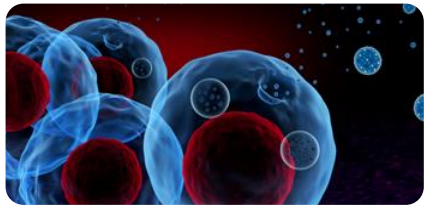
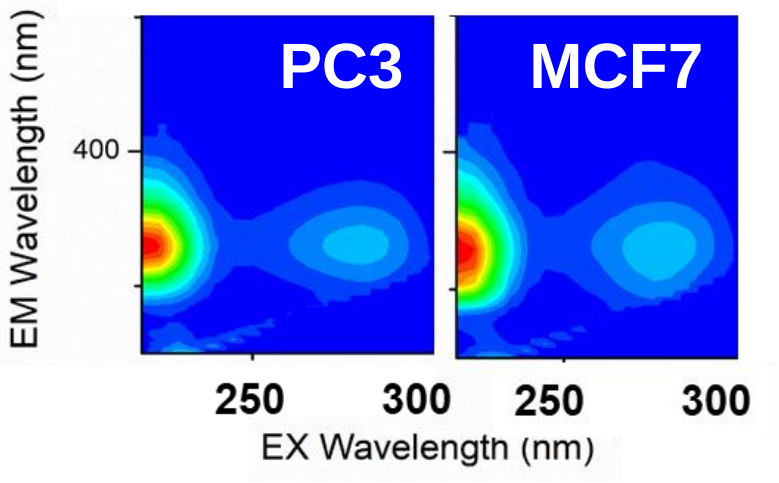
Exosomes



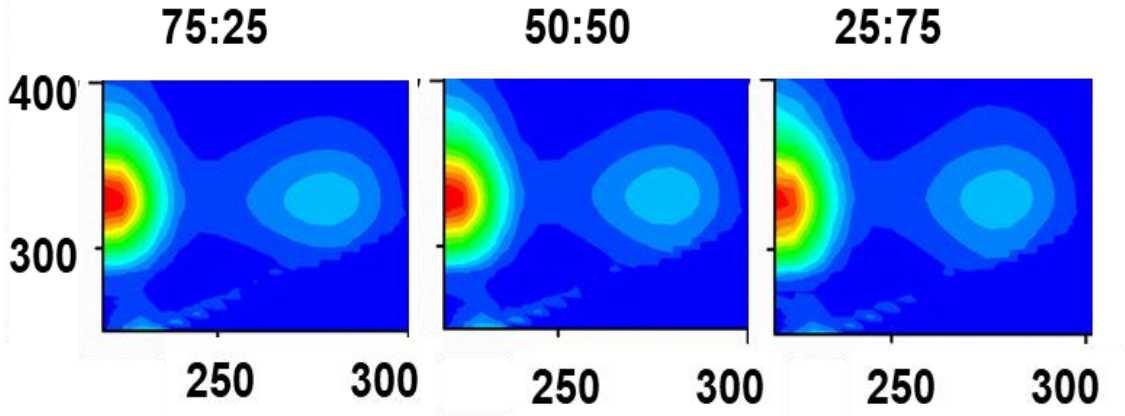
Pure Exosome Samples

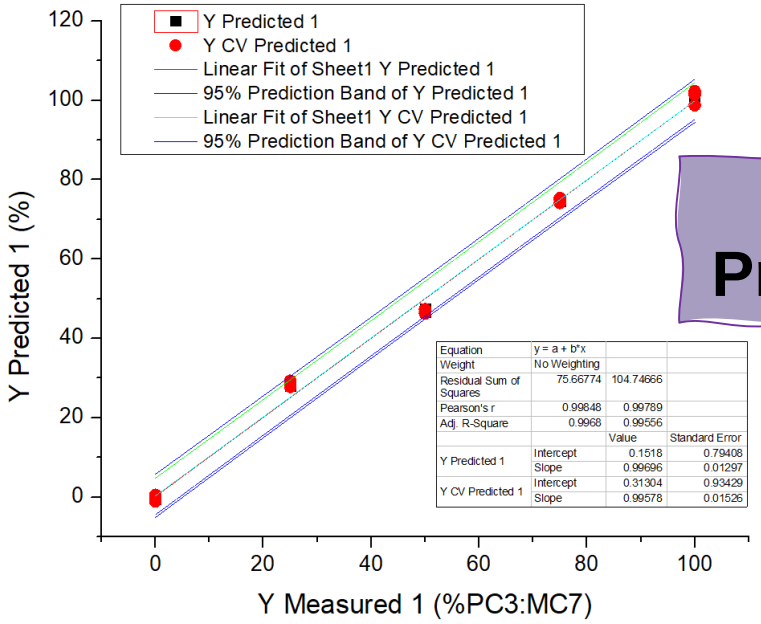


Rapid Screening

- ✓ Quantify exosome mixtures
- ✓ Resolve exosome types

Mixture of PC3 & MCF7





Linear Prediction

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Deployment into Pharma PAT

A-TEEM on Aqualog Platform - now

- **R&D tools with options for process**

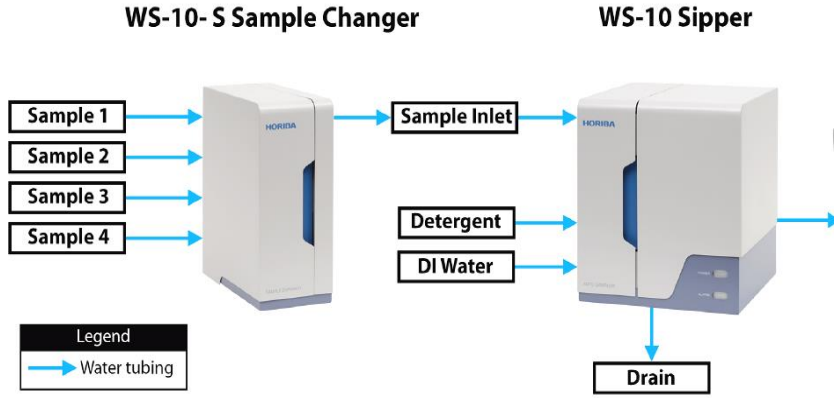
Analyzer with “A-TEEM Inside”

- **Manufacturing environment**

Batch sampling

Unattended
multi-sample measurements

Sipper for on-line sampling



For Periodic in-Process

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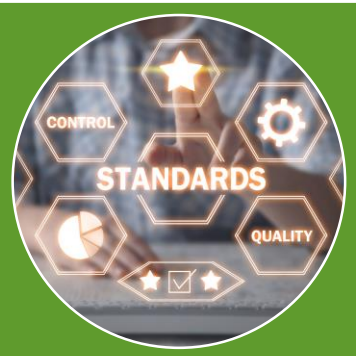
Conclusion



Quality Control

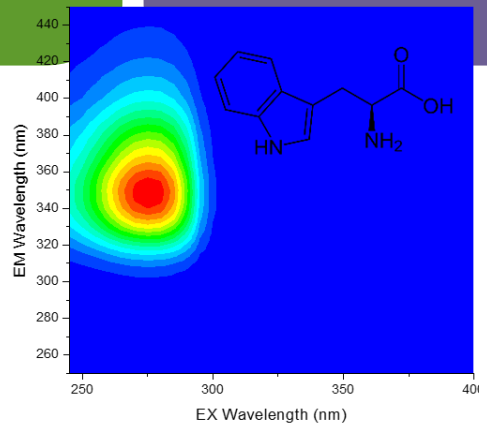
- Contaminants detection
- Identification of Unknowns
- Protein structure & stability
- Cell culture media changes

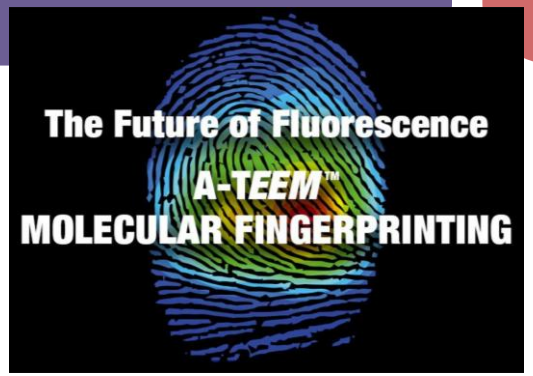
Quantification

- AAV full vs empty ratio
- Binding events – using tyrosine environment as marker

Differentiation & Classification

- Exosomes types differentiation
- Multi-components Vaccines
- AAVs serotype differentiation





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