Exploring the Multidimensionality of High-Resolution Photoluminescence Spectroscopy to Face Environmental Challenges

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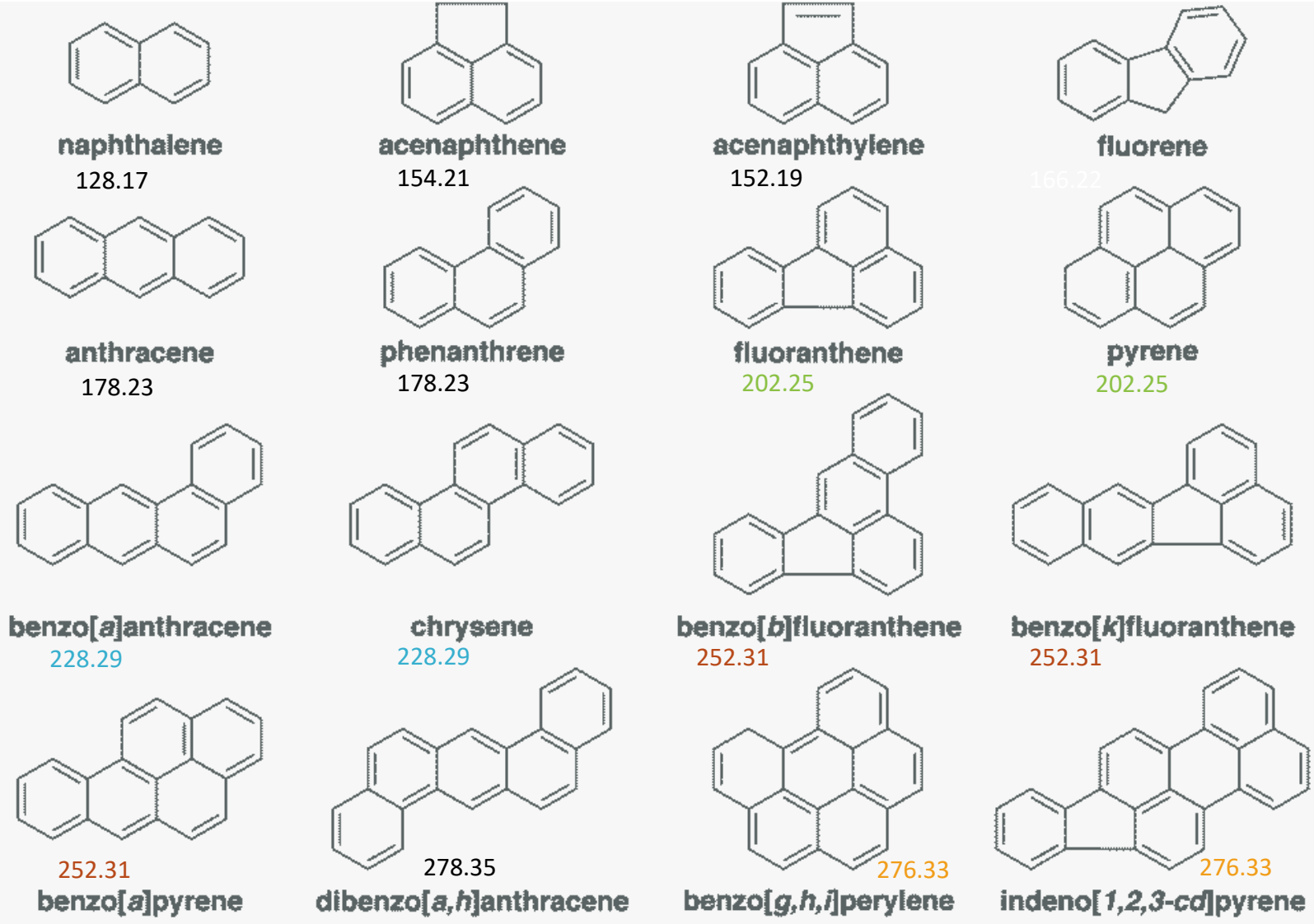
Polycyclic Aromatic Hydrocarbons (PAHs)

- PAHs are formed during incomplete combustion or pyrolysis of organic matter containing carbon and hydrogen.
- Since combustion of organic materials is involved in countless natural processes or human activities, PAHs are omnipresent and abundant pollutants in air, soil and water.
- Many PAHs have shown to be mutagens and/or potent carcinogens in laboratory animals assays.
- *The US Environmental Protection Agency (EPA) enforces the routine monitoring of sixteen PAHs in air, soil and water.*

EPA-PAHs



128.17 g.mol^{-1} ≤ Molecular Weight ≤ 278.35 g.mol^{-1}

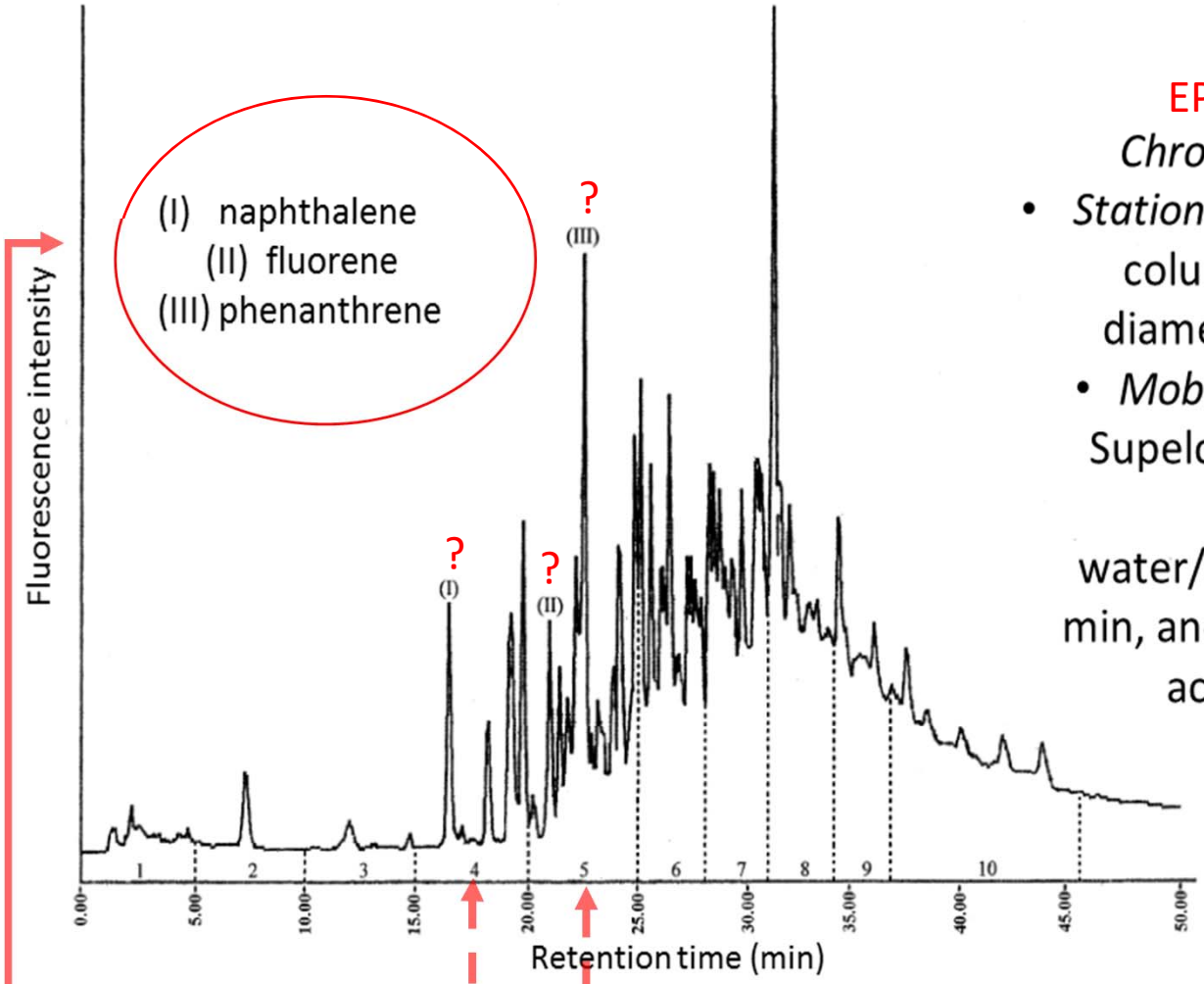
Classic Approaches to the Analysis of EPA-PAHs

- Relatively simple samples:
  - ➢ HPLC with UV-vis absorption and/or fluorescence detection

  or

  - ➢ GC-MS
- Complex samples:
  - ➢ Analysis should be made with two “intrinsically different” methods

HPLC chromatogram of an API (American Petroleum Institute) water extract



EPA Method 550.1: SPE-HPLC

Chromatographic conditions

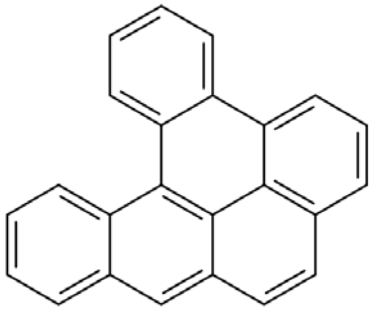
- *Stationary phase:* Supelcosil LC-PAH column (25 cm length, 4.6 mm diameter, and 5 µm particle size.
- *Mobile phase:* As stipulated by Supelco; i.e. 1.5mL/min flow rate, isocratic elution with water/acetonitrile (60:40 v/v) for 5 min, and then linear gradient to 100% acetronitrile over 20 min.

EPA Method 525.3
SPE-HRGC/MS

High-Molecular Weight PAHs (HMW-PAHs)

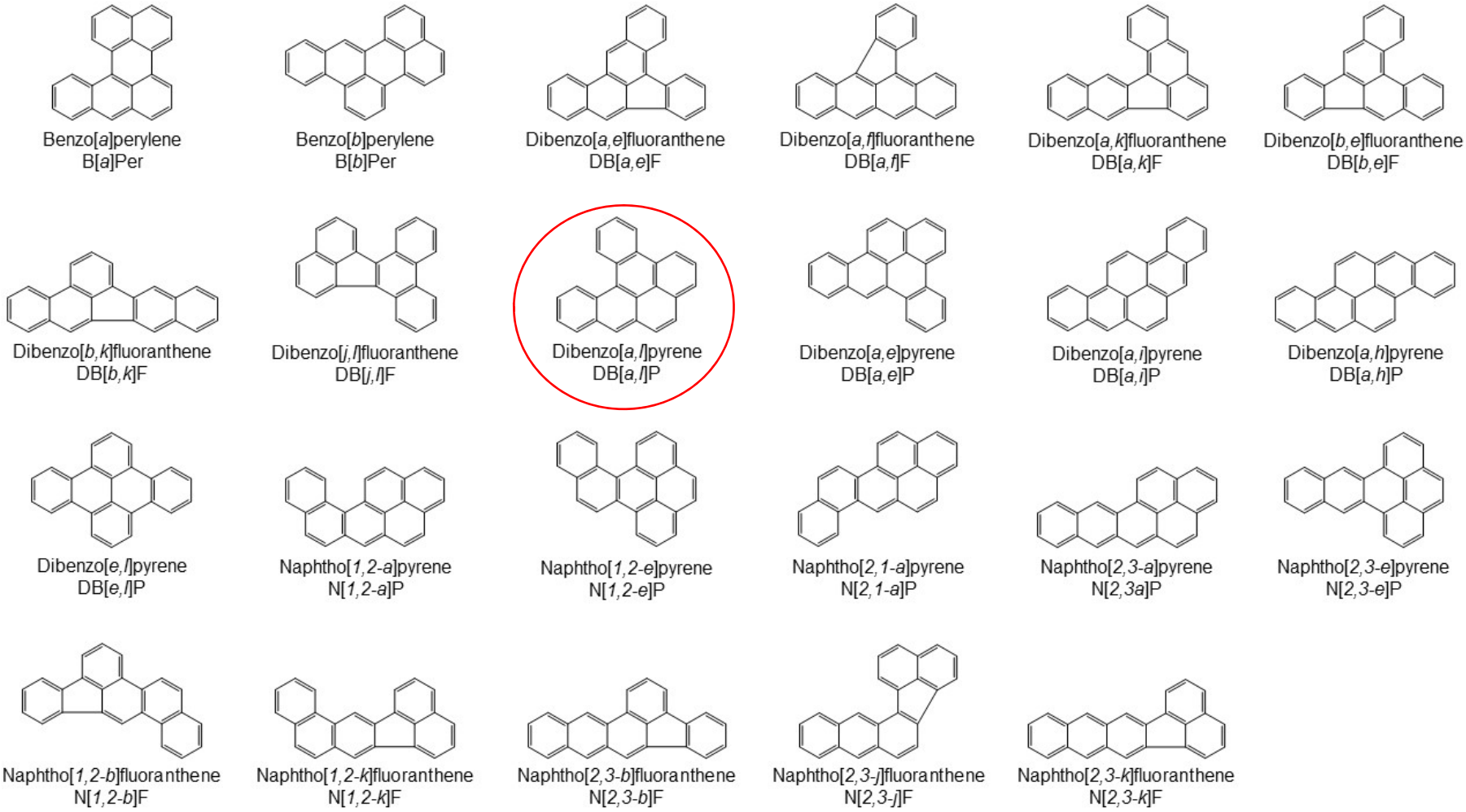
- EPA-PAHs are only a small fraction of the PAHs that are known to exist in environmental samples.
- Of particular concern is the omission of high molecular weight PAHs (HMW-PAHs), *i.e*. PAHs with molecular weight (MW) greater than 300 $g.mol^{-1}$.
- Biological activities of HMW-PAHs have revealed high mutagenic response when isolated from environmental and combustion-related samples.
- A crucial example is dibenzo[*a,l*]pyrene (DB[a,l]P, MW ≈ 302 $g.mol^{-1}$) which is the most potent carcinogenic PAH yet discovered.
- Its toxicity is approximately 100 times higher than that of benzo[*a*]pyrene, which is the most potent carcinogenic in the EPA list.

Why isn't DBalP included in the EPA list?

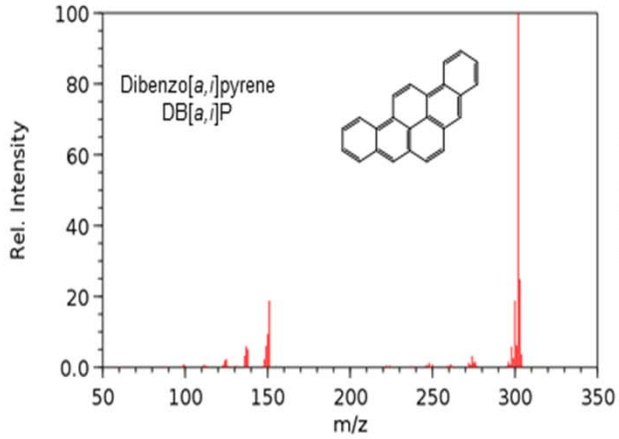


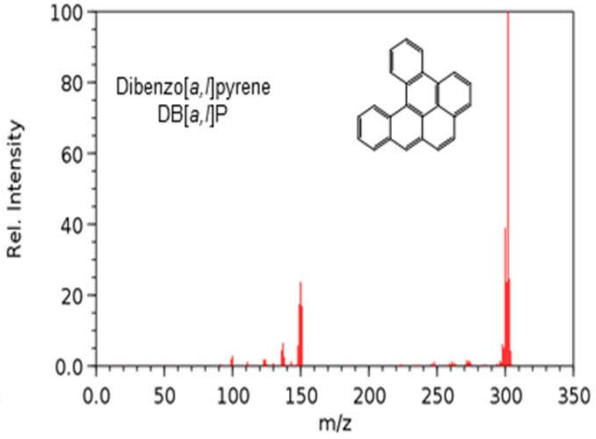
DBalP

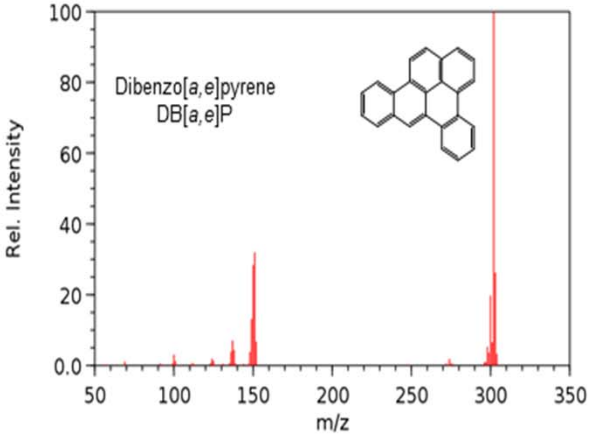
- There are 88 possible isomers with molecular mass 302Da.
- Only 23 of those isomers are commercially available.
- Those 23 isomers have very similar chromatographic behaviors and almost identical mass fragmentation patterns.

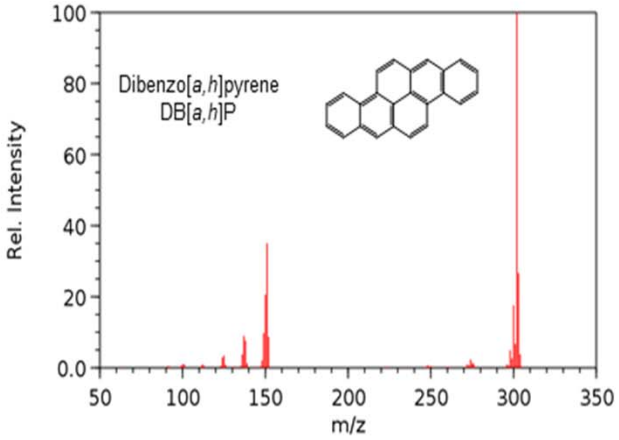


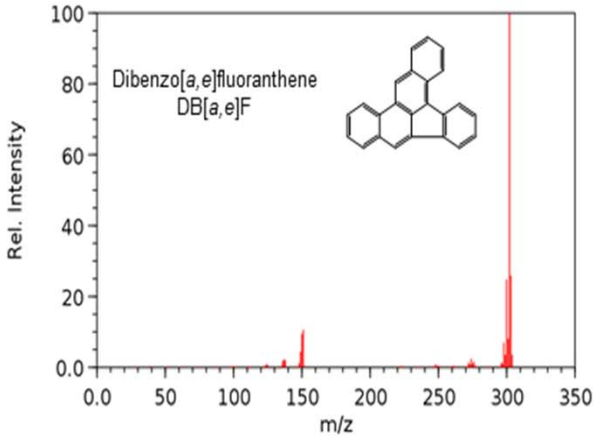
Electron ionization mass spectra of PAHs with MW = 302 Da.

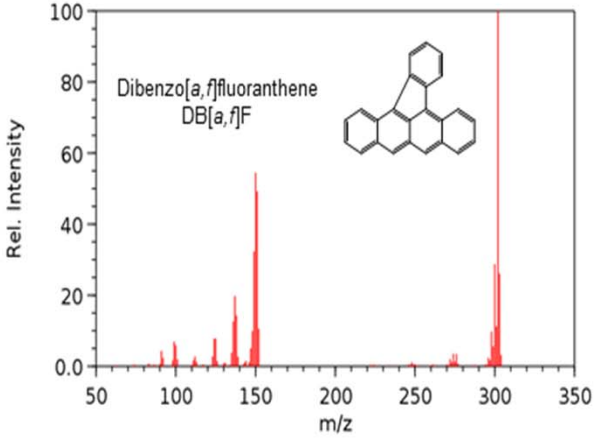












Recent Chromatographic Developments

Normal-phase liquid chromatography (NPLC) with UV-VIS Absorption Detection

GC-MS of NPLC fractions

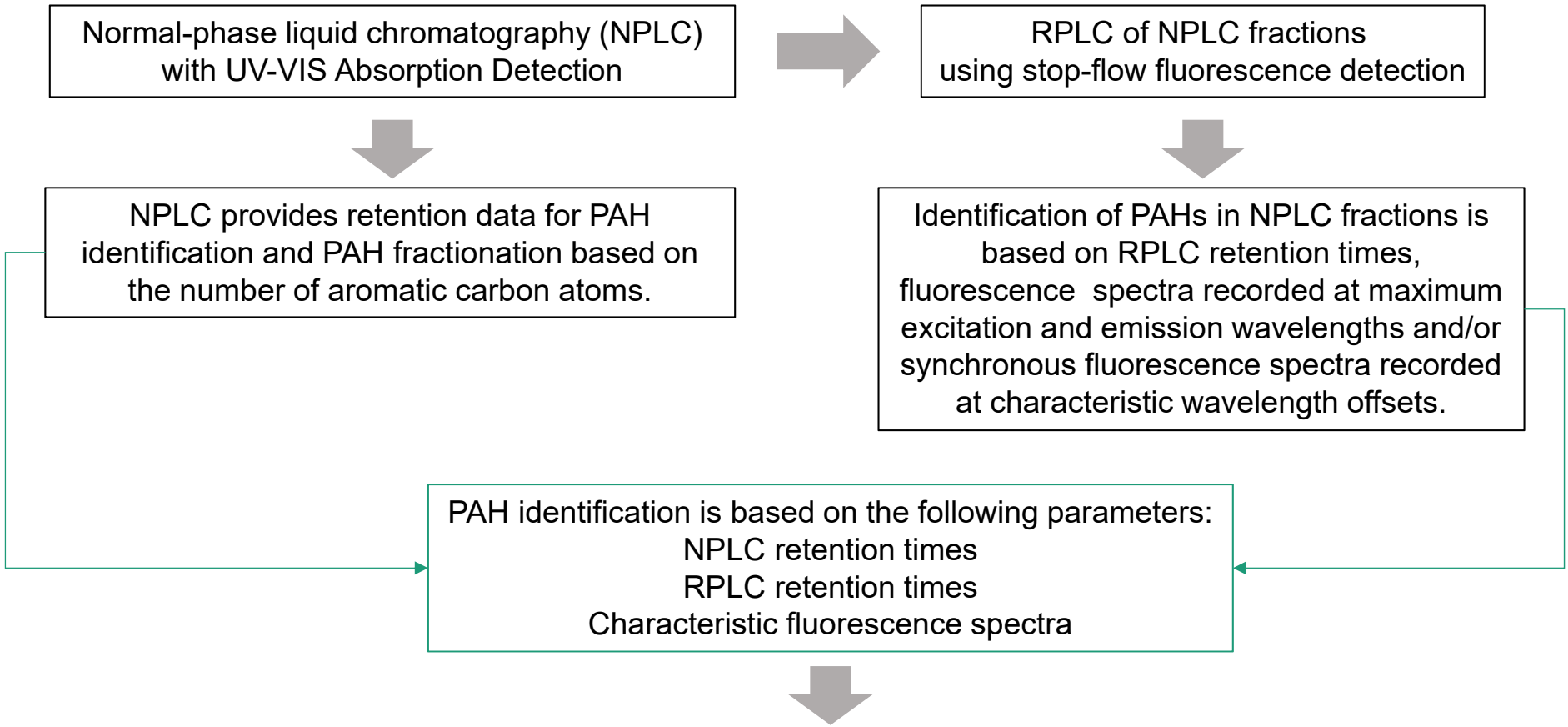
NPLC provides retention data for PAH identification and PAH fractionation based on the number of aromatic carbon atoms.

Identification of PAHs in NPLC fractions is made via GC-MS. Identification is based on GC retention times and predominant mass ion peak.

PAH identification is based on the following three parameters:
NPLC retention times
GC retention times
Predominant mass ion peaks

Standard Reference Material	Description
SRM 1597a	Coal tar
SRM 1991	Coal tar/Petroleum extract
SRM 1975	Diesel particulate extract

W. B. Wilson, H. V. Hayes, L. C. Sander, A. D. Campiglia, S. A. Wise, Analytical Bioanalytical Chemistry, 409, 5171, 2017.
W. B. Wilson, H. V. Hayes, L. C. Sander, A. D. Campiglia, S. A. Wise, Analytical Bioanalytical Chemistry, 409, 5291, 2017.



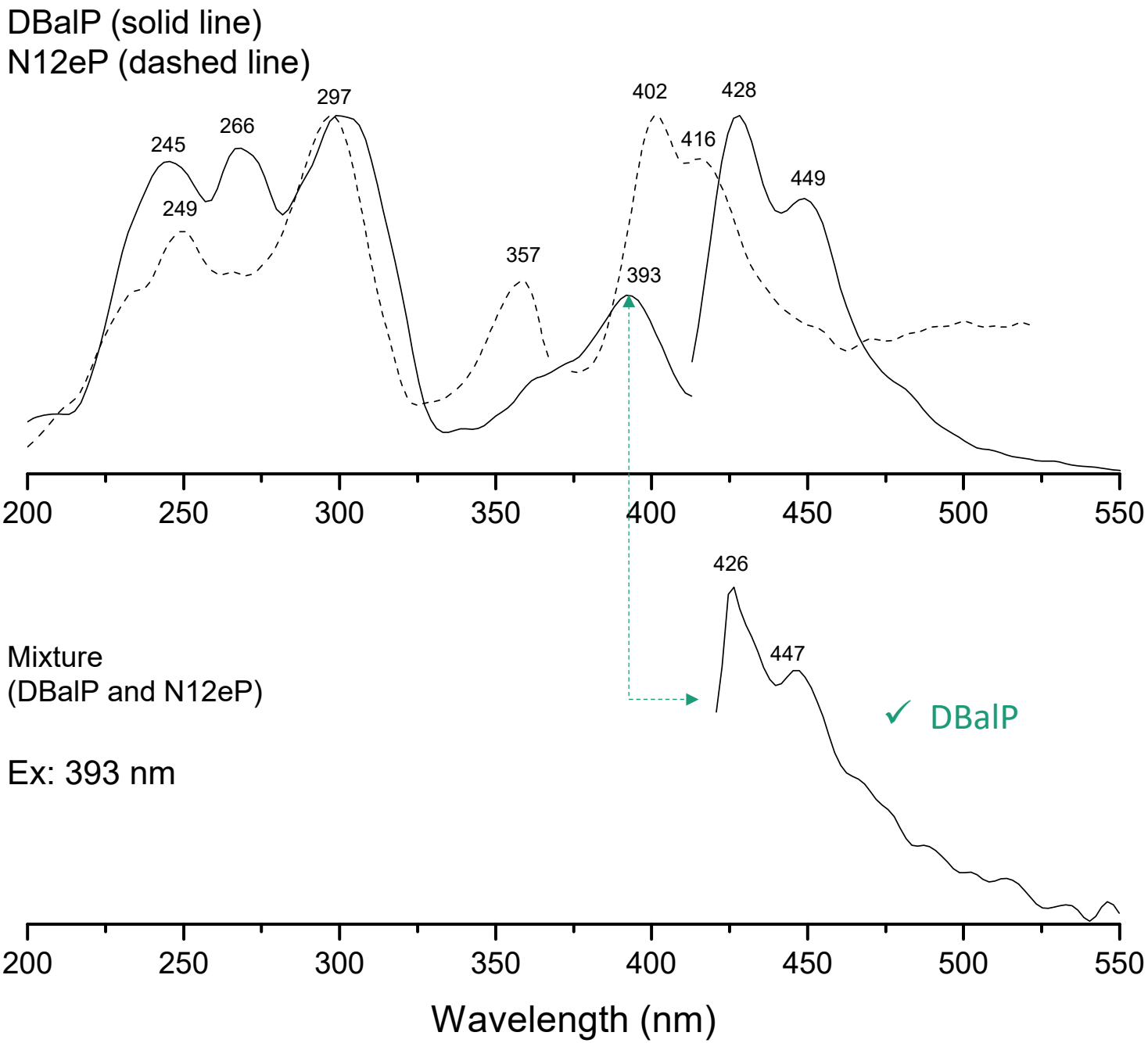
Standard Reference Material	Description
SRM 1597a	Coal tar

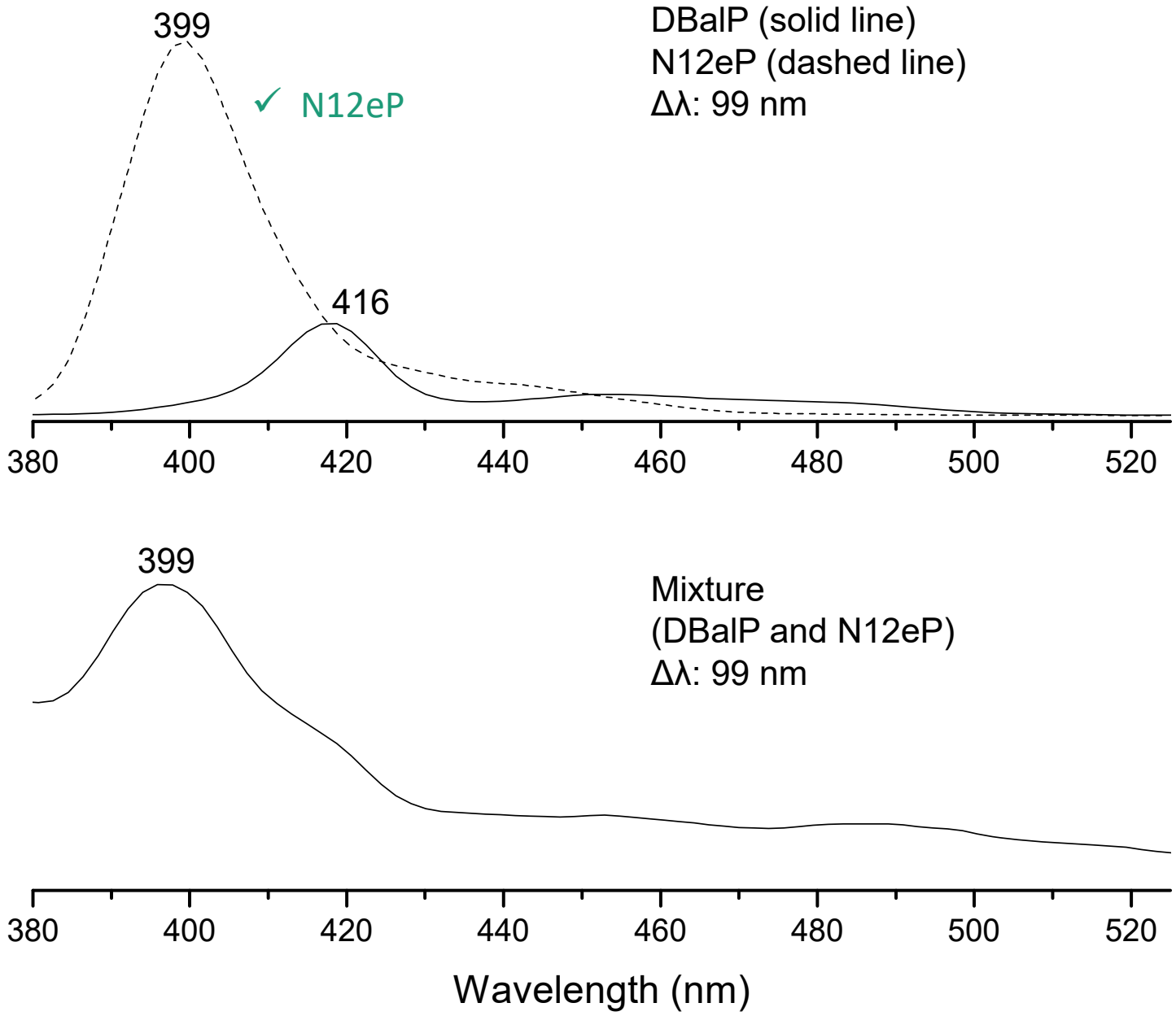
H. V. Hayes, W. B. Wilson, A. M. Santana, A. D. Campiglia, L. C. Sander, S. A. Wise, Microchemical Journal, 149, 104061, 2019.

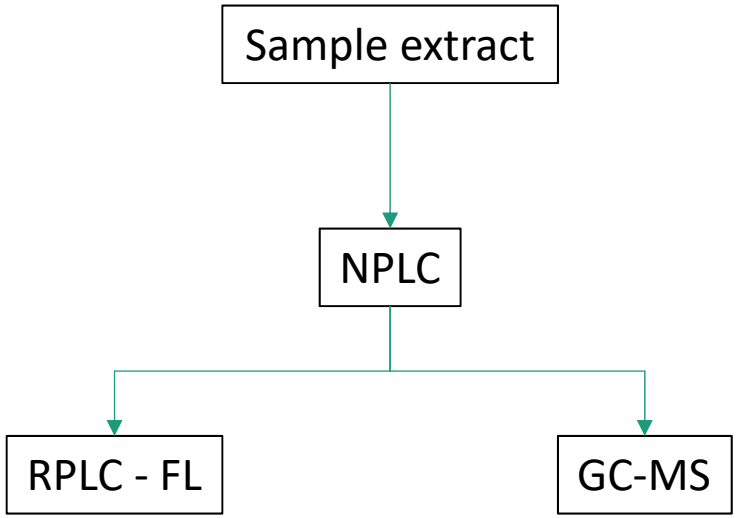
H. V. Hayes, W. B. Wilson, L. C. Sander, S. A. Wise, A. D. Campiglia, Analytical Methods, 10, 5051, 2018.

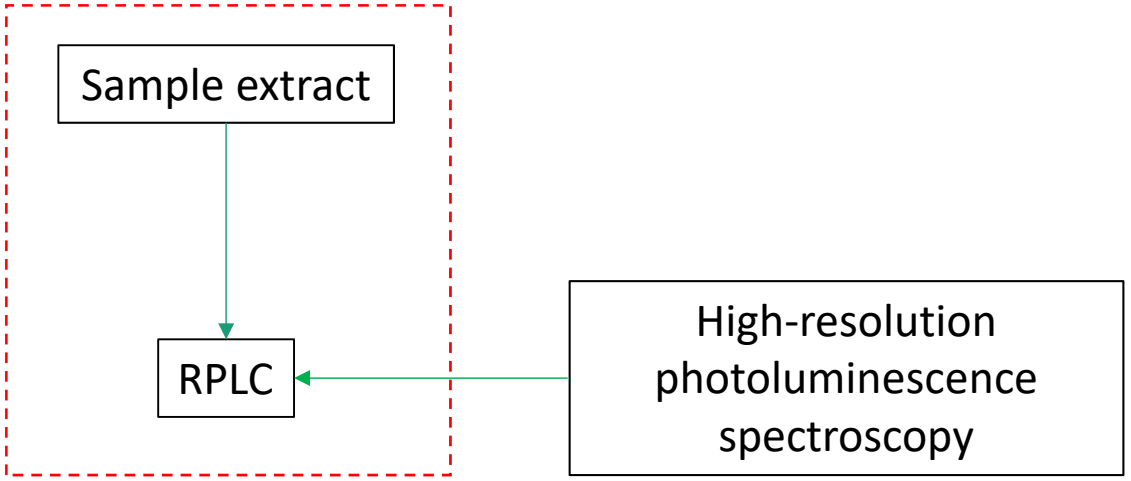
Retention Times for the RPLC Determination of MM 302 Da PAH Isomers in NPLC Fractions

PAH	RPLC Retention Time (min)
NPLC Fraction 10	Fraction 10
BaPer	7.76 ± 0.12
DBalP	8.41 ± 0.17
N12eP	8.53 ± 0.29
N12aP	14.05 ± 0.09
NPLC Fraction 11	Fraction 11
DBbeF	10.59 ± 0.15
DBaiP	42.45 ± 0.51
N23aP	48.10 ± 0.33
DBahP	58.30 ± 0.89
NPLC Fraction 12	Fraction 12
DBjlF	10.81 ± 0.17
N12bF	11.88 ± 0.03
DBakF	12.75 ± 0.07
N23eP	13.57 ± 0.07
DBaeP	14.83 ± 0.15
N23jF	15.14 ± 0.04
N12kF	15.52 ± 0.13
BbPer	16.34 ± 0.14
DBelP	17.01 ± 0.12
DBbkF	21.75 ± 0.24
N23bF	22.80 ± 0.23
N21aP	29.90 ± 0.03
N23kF	55.49 ± 0.36


DBalP (solid line)
N12eP (dashed line)
245
249
266
297
357
393
402
416
428
449
200
250
300
350
400
450
500
550
Mixture
(DBalP and N12eP)
Ex: 393 nm
426
447
✓ DBalP
Wavelength (nm)


399
✓ N12eP
416
DBalP (solid line)
N12eP (dashed line)
Δλ: 99 nm
380
400
420
440
460
480
500
520
399
Mixture
(DBalP and N12eP)
Δλ: 99 nm
380
400
420
440
460
480
500
520
Wavelength (nm)

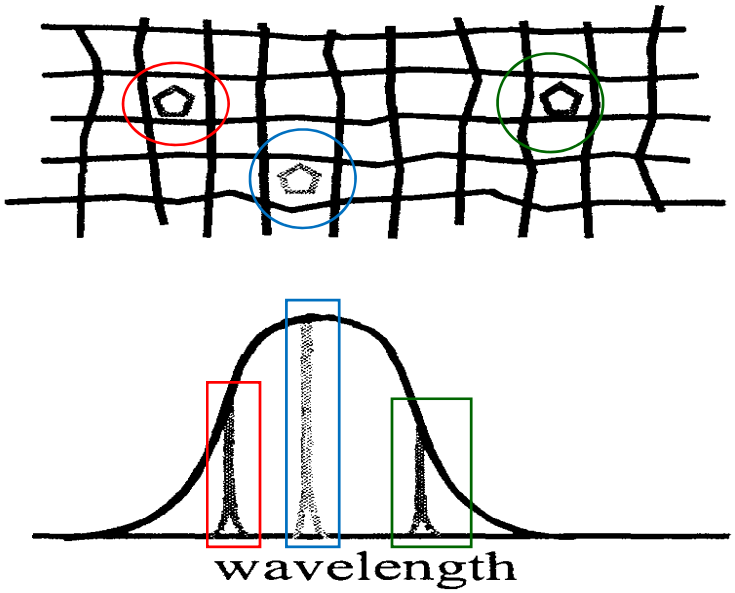




EPA Method 550.1

Main Limitation of Room-Temperature Photoluminescence Spectroscopy

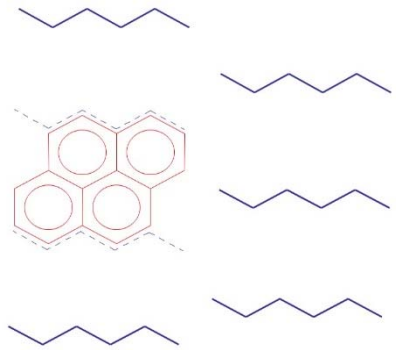
- The main limitation of photoluminescence spectroscopy towards the selectivity of analysis is the broad nature of room-temperature excitation and emission spectra.
- The diffuse character of such spectra limits the information content – i.e., vibrational information - and promotes spectral overlapping in the analysis of fluorophore mixtures.
- The full-width at half maximum (FWHM) of an absorption or emission band results from the contribution of homogeneous and inhomogeneous band-broadening.
  - ***Homogeneous broadening*** is related to the lifetime of the transition.
  - ***Inhomogeneous band-broadening*** results from the fluorophore exposure to different chemical environments in the analytical sample.

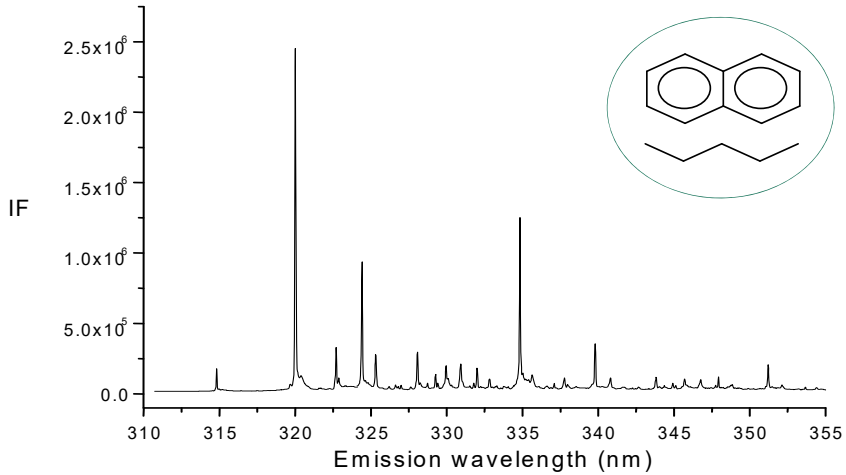


The key to spectral narrowing is to minimize inhomogeneous band-broadening

Shpol'skii Spectroscopy

- Sample is maintained at cryogenic temperature to ensure that most of the population of PAH molecules in the ground state (S_0) is in the lowest vibrational level of S_0.
- The solvent should form a poly-crystalline matrix at low temperature.
- Weak guest-host interactions, where the guest is the PHA and the host is the solvent.
- In the ideal situation, a PAH molecule displaces a distinct number of solvent molecules in the lattice and fits in a well defined manner in the available space.
- As a result, individual PAH molecules experience the same interaction with the regular matrix and, therefore, their S_0-S_1 energy differences are identical.





Our Contributions to Shpol'skii Spectroscopy

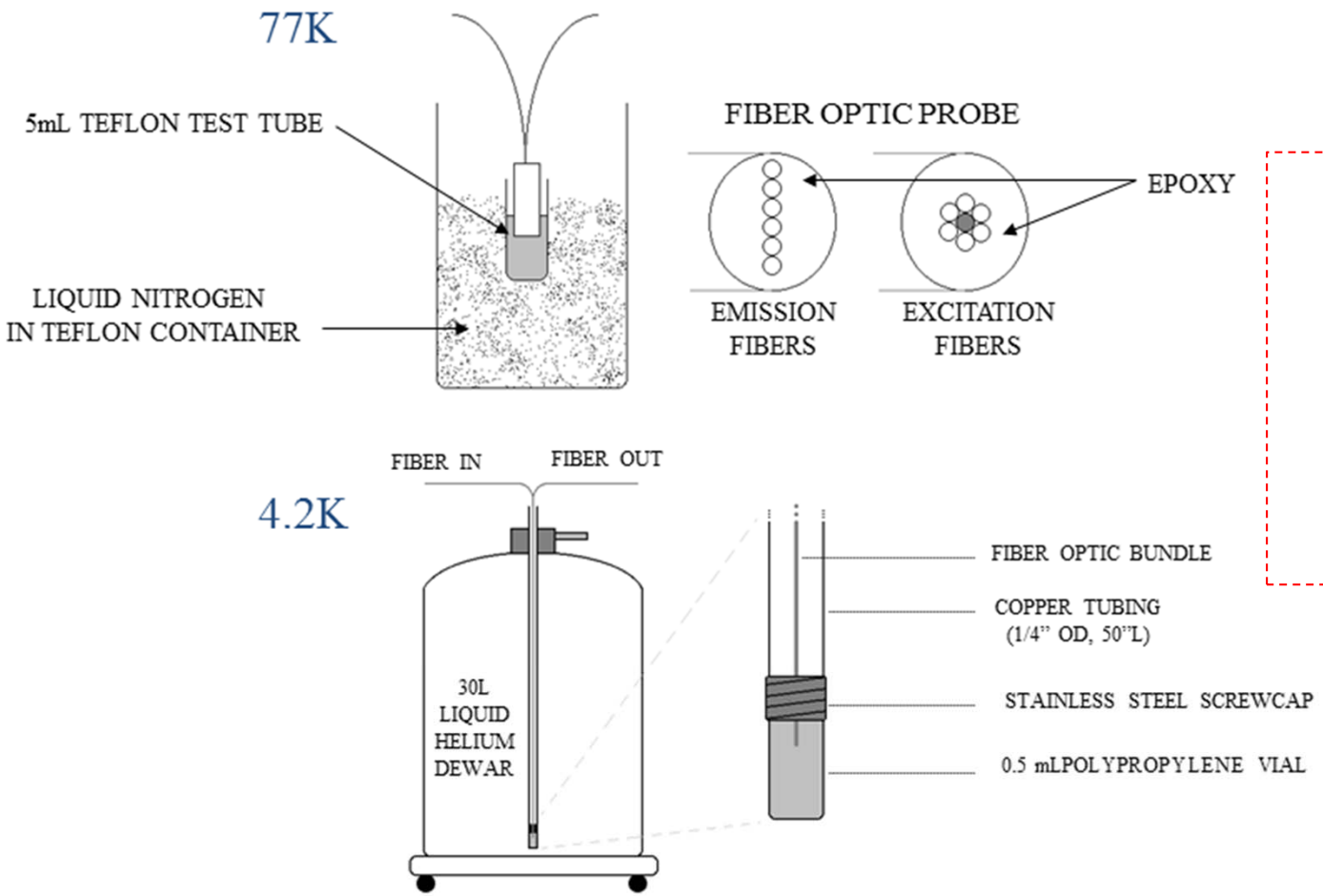
Limitations

- Inconvenient sample freezing procedures.
- Poor reproducibility of measurements.
- Slow spectral acquisition.
- Photo-degradation upon sample excitation.
- No qualitative parameter for spectral confirmation and peak purity.

Improvements

- Fiber optic probes for cryogenic measurements at liquid nitrogen (77K) and liquid helium (4.2K) temperatures.
- Instrumental system for real-time collection of multidimensional data formats containing spectral and lifetime information.

Cryogenic Fiber Optic Probes



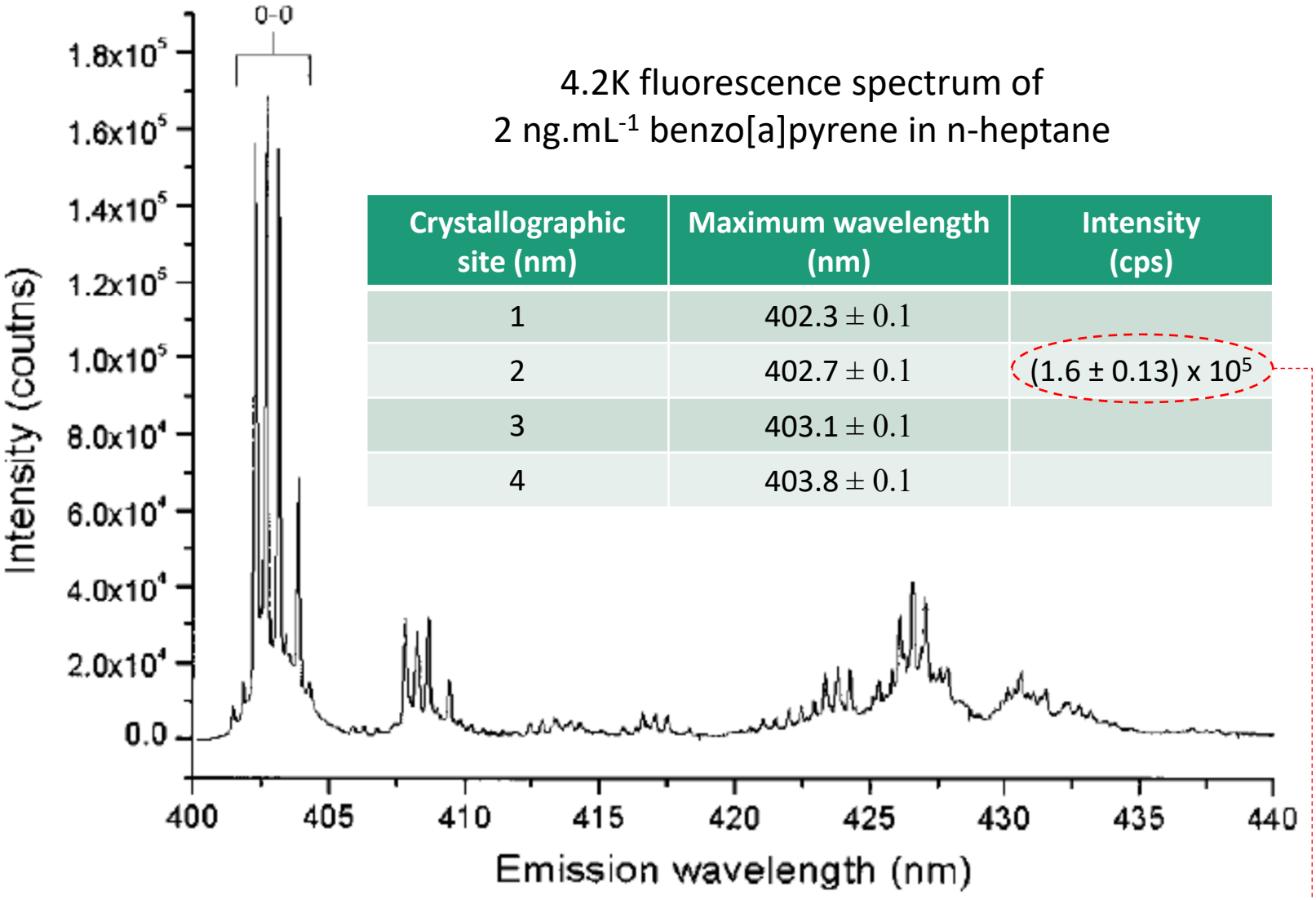
- No interfaces to scatter radiation.
- No humidity or bubbling artifacts.
- Simplicity of dunking the sample into the liquid cryogen.

A. J. Bystol, A. D. Campiglia and G. D. Gillispie, *Applied Spectroscopy*, 54, 910, 2000.

A. J. Bystol. A. D. Campiglia and G. D. Gillispie, *Analytical Chemistry*, 73, 5762, 2001.

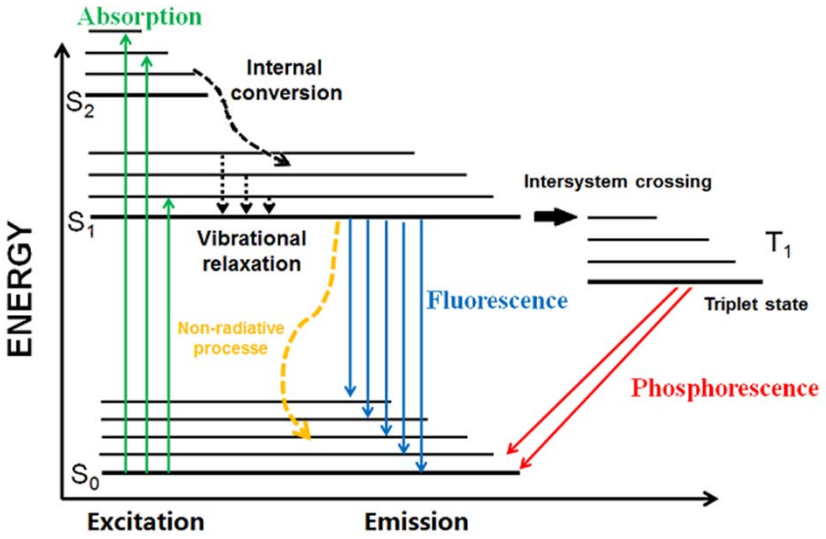
4.2K fluorescence spectrum of
2 ng.mL^{-1} benzo[a]pyrene in n-heptane

Crystallographic site (nm)	Maximum wavelength (nm)	Intensity (cps)
1	402.3 ± 0.1	
2	402.7 ± 0.1	(1.6 ± 0.13) x 10^5
3	403.1 ± 0.1	
4	403.8 ± 0.1	

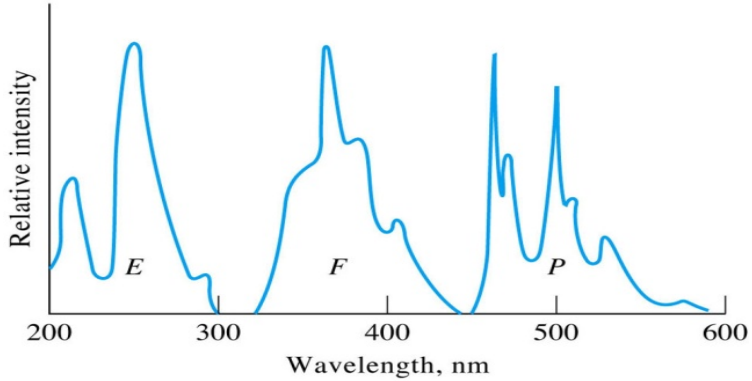


Relative standard deviations of signal intensities ≤ 8%

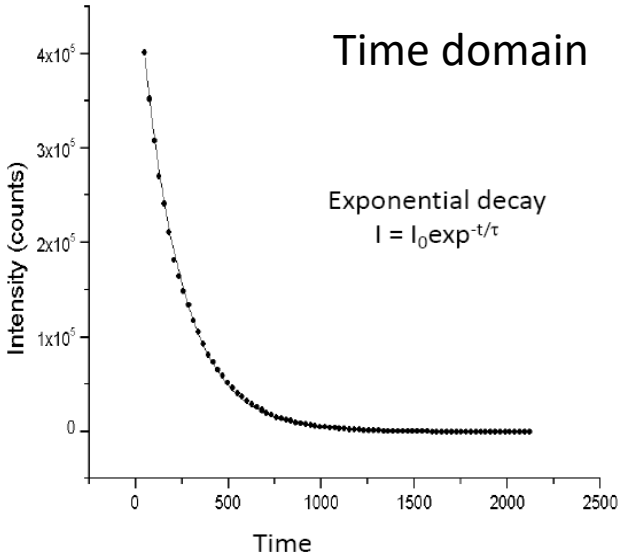
Multidimensionality of Photoluminescence Spectroscopy



Wavelength domain

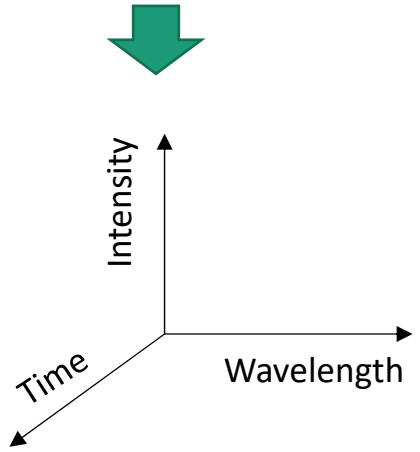


Time domain

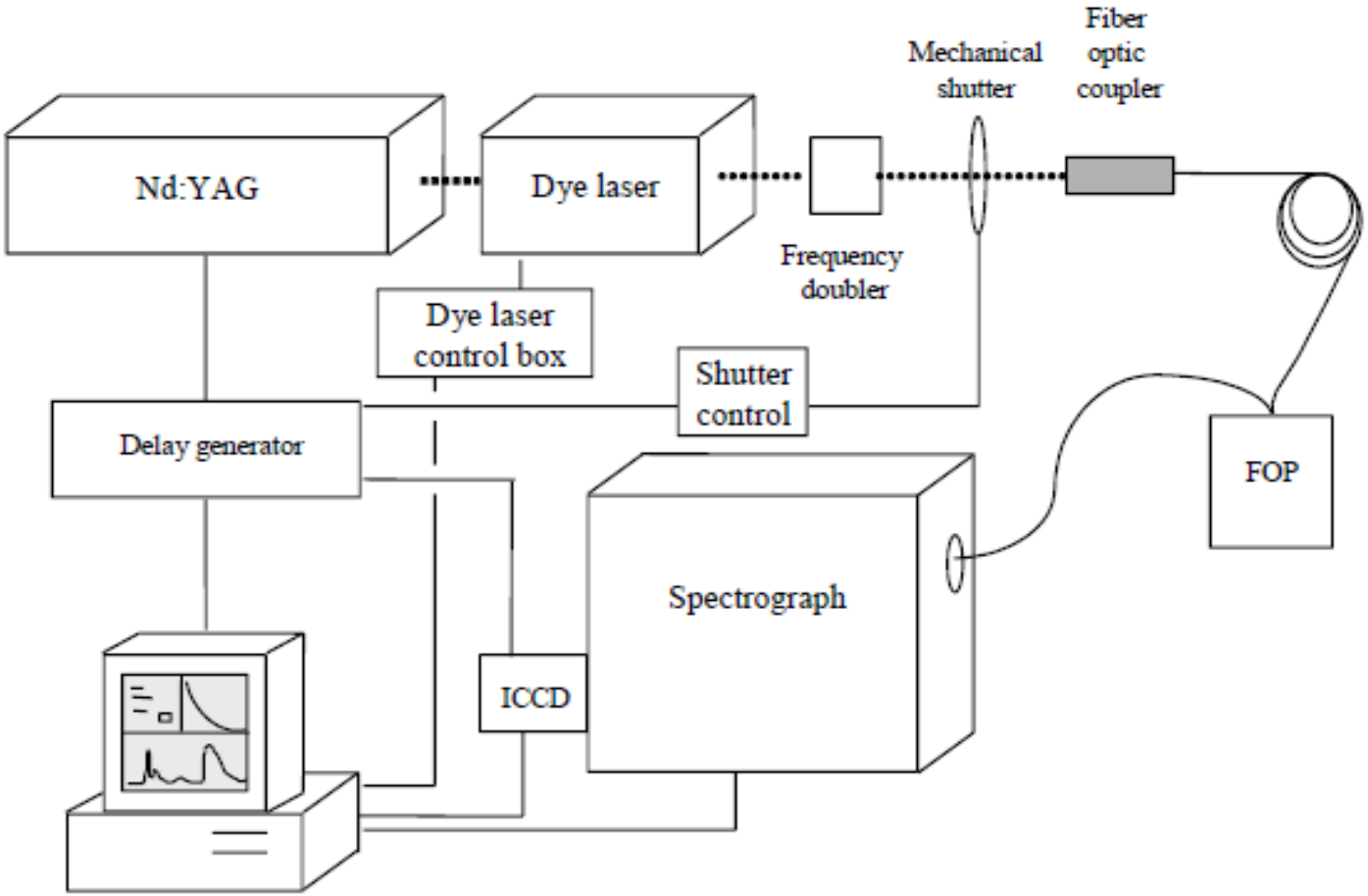


Exponential decay
$I = I_0 \exp^{-t/\tau}$

- Fluorescence
  μs or shorter
- Phosphorescence
  ms or longer



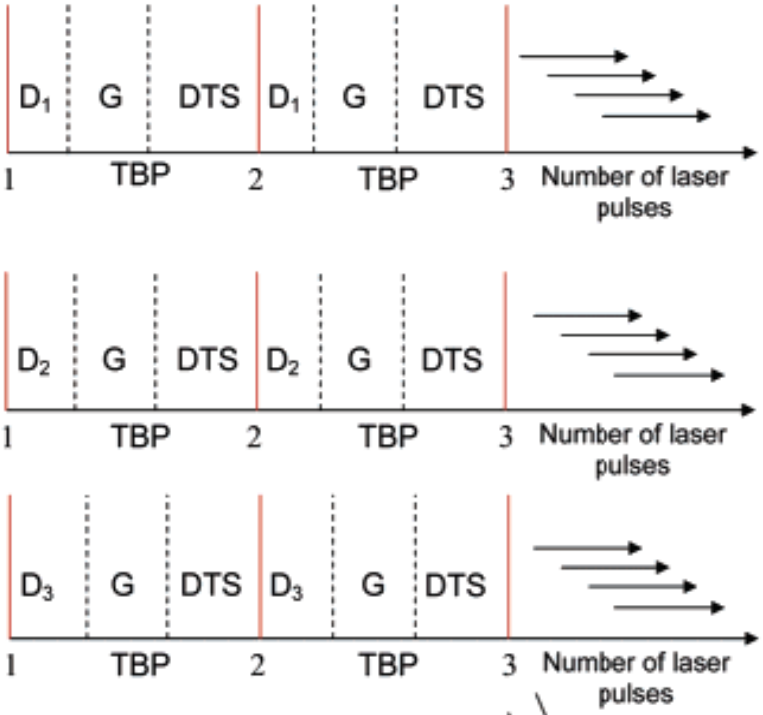
Multidimensional Luminescence System

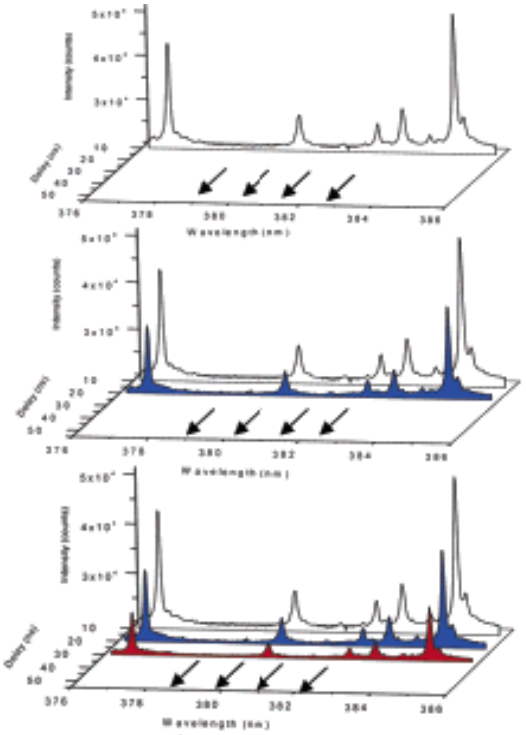


Unique capabilities

- Wavelength time matrices (WTMs)
- Time-resolved excitation emission matrices (TREEMs)

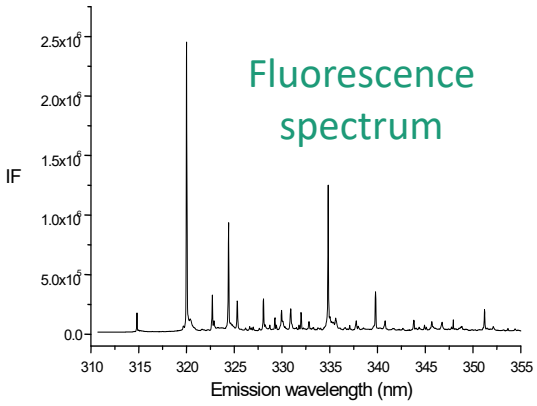
Recording a Wavelength Time Matrix(WTM)



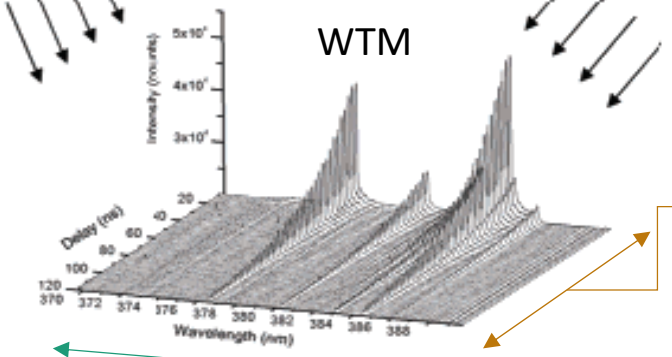


$D_1 < D_2 < D_3 < \ldots$
$G = \text{constant}$

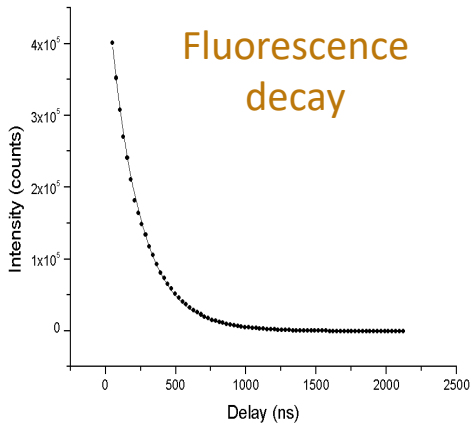
Fluorescence spectrum



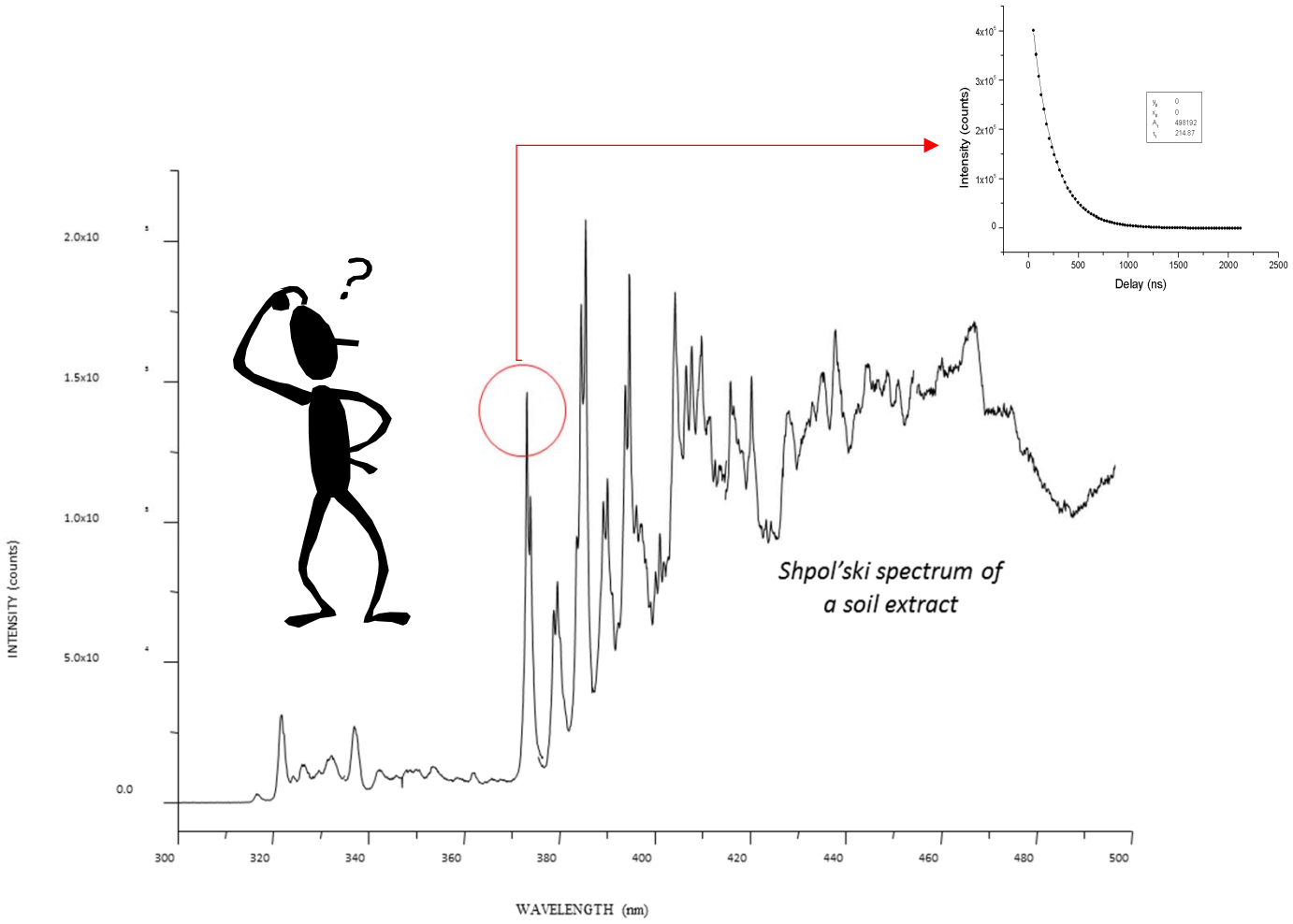
WTM



Fluorescence decay

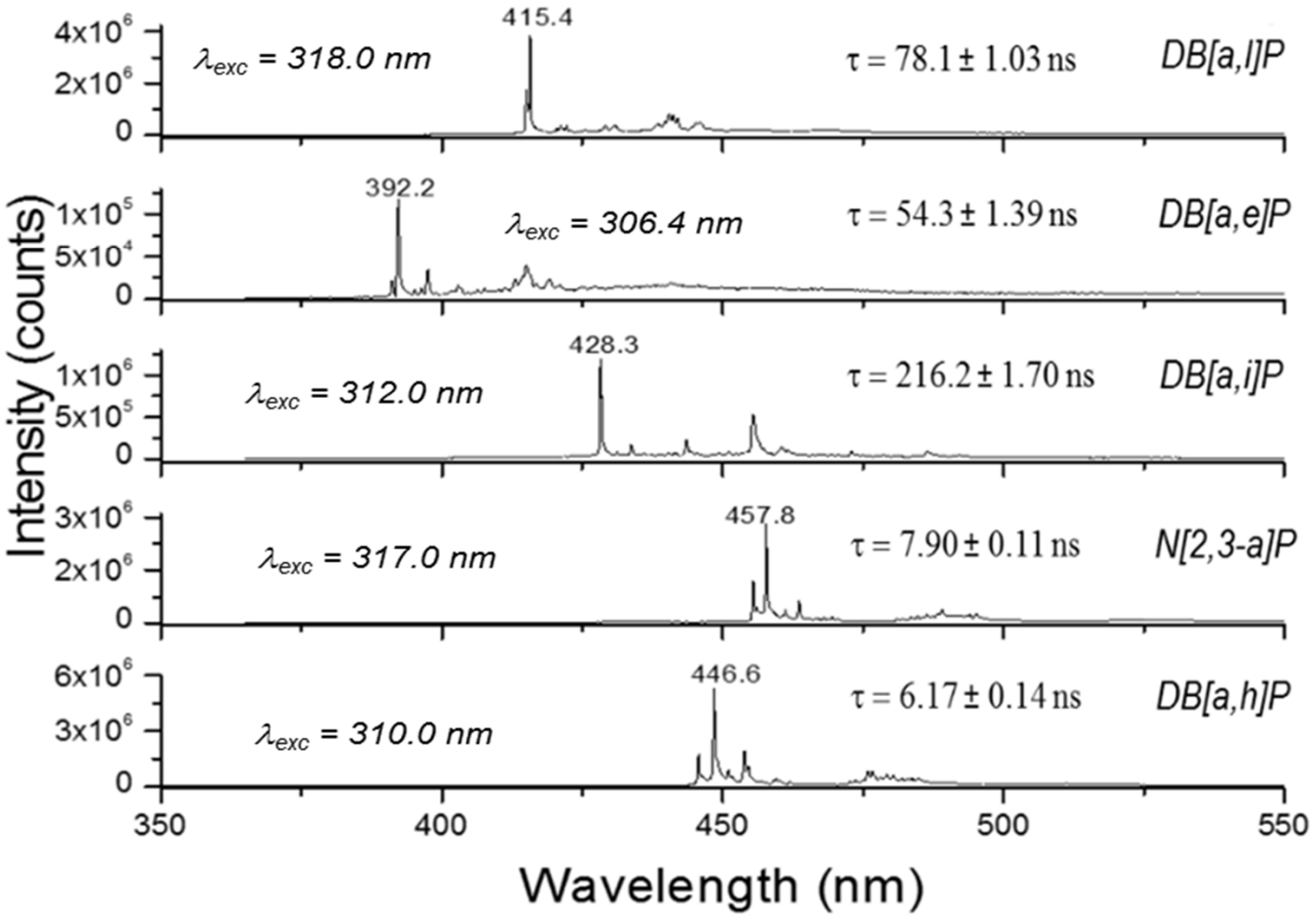


Advantages of recording fluorescence lifetimes



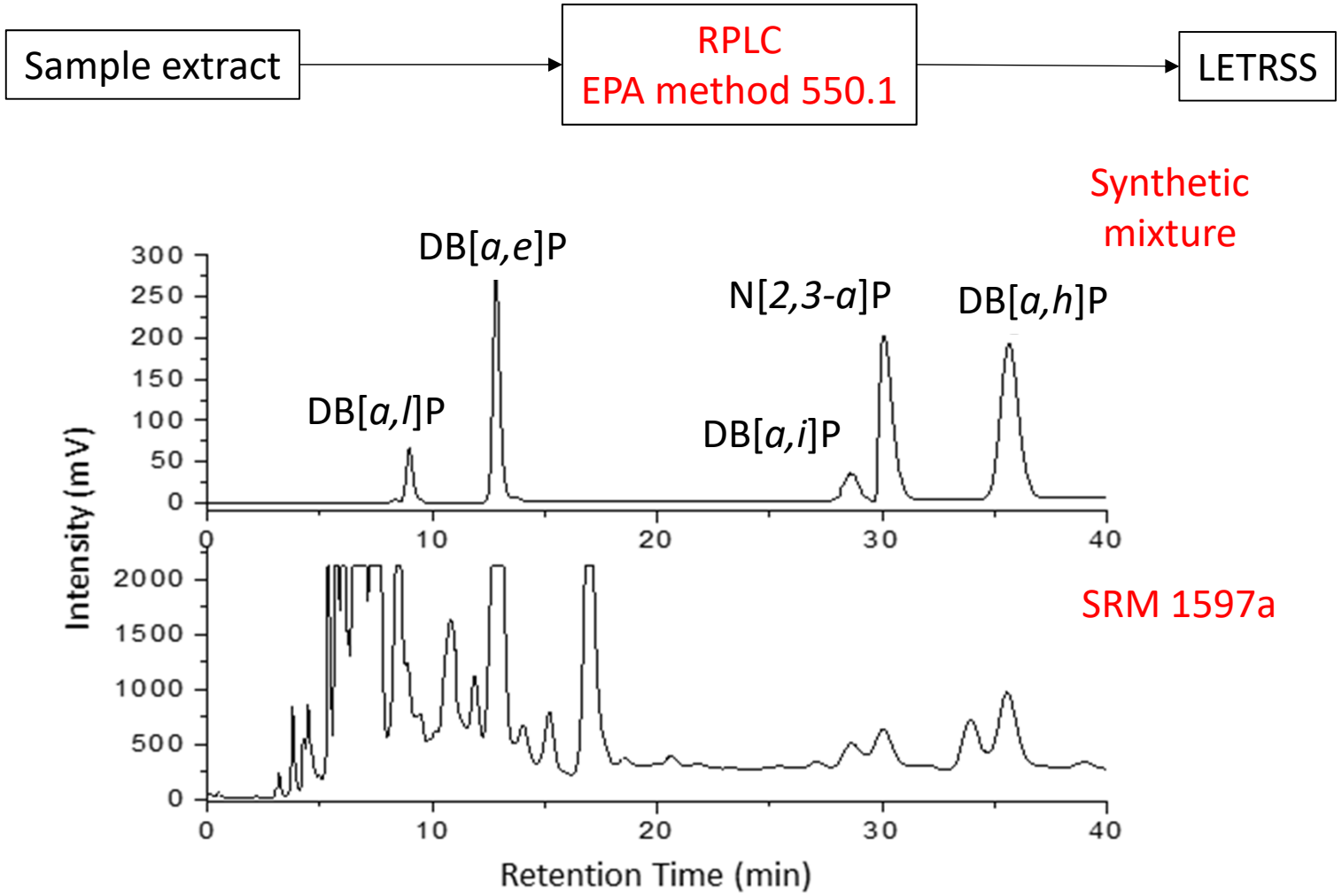
- It provides an additional parameter for qualitative analysis
- Single exponential decays report on spectral peak purity, i.e. an essential condition for the accurate quantitative determination of PAHs in complex environmental matrixes.

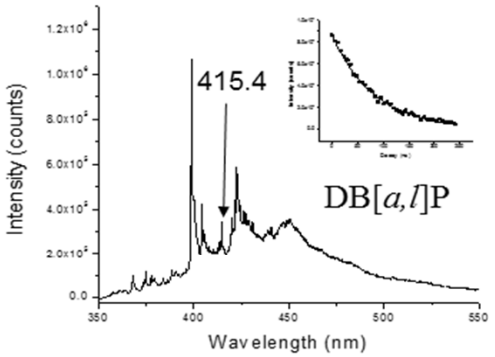
Is LETRSS Able to Differentiate PAH Isomers with MM 302Da?

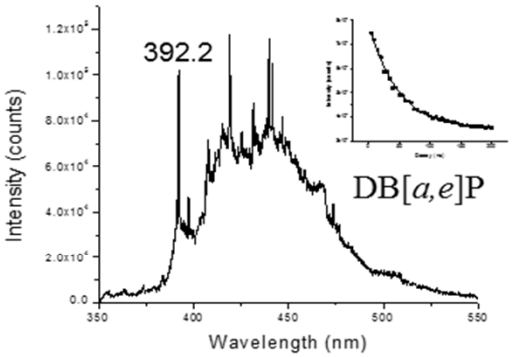


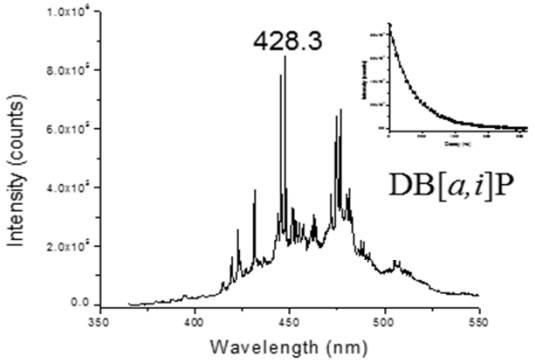
4.2K WTMs were recorded under site-selective excitation.

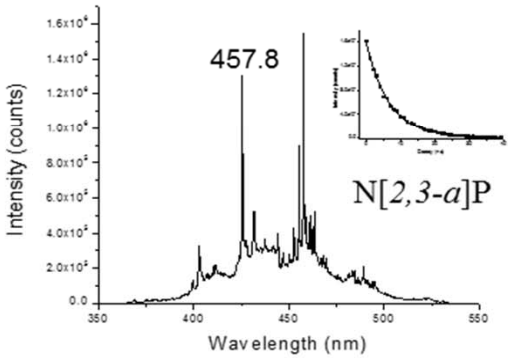
RPLC-LETRSS Analysis of SRM 1597a

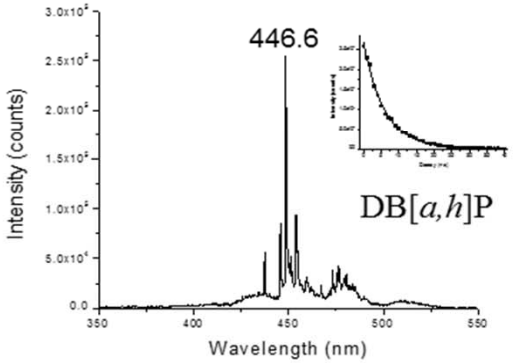












4.2K Fluorescence Lifetimes

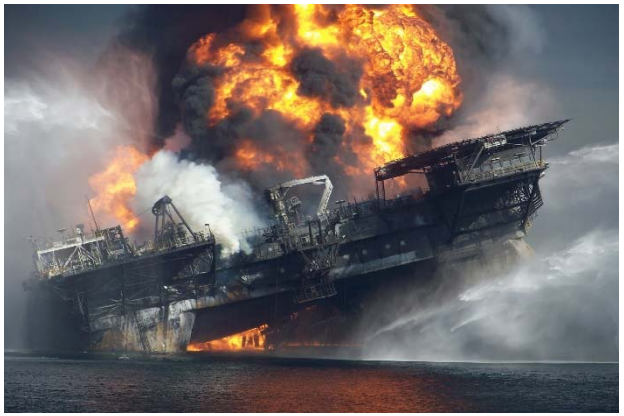
HMW-PAH	*Standard*	*SRM 1597a*
DB[*a,l*]P	78.1 ± 1.03	80.4 ± 2.73
DB[*a,e*]P	54.3 ± 1.39	53.4 ± 0.64
DB[*a,i*]P	212.0 ± 2.20	211.1 ± 1.17
N[*2,3-a*]P	6.83 ± 0.05	7.13 ± 0.23
DB[*a,h*]P	6.31 ± 0.09	6.14 ± 0.10

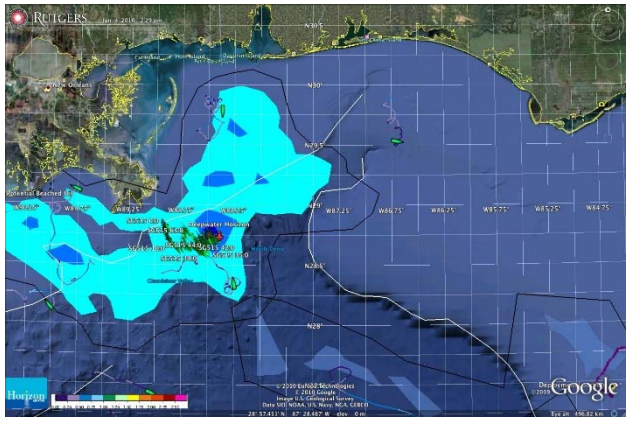
HMW-PAH	OR (%)	LDR ($ng.mL^{-1}$)	R	LOD ($ng.mL^{-1}$)	RSD (%)	NIST ($mg\ Kg^{-1}$)	Experimental [g] ($mg\ Kg^{-1}$)
DB[a,l]P	98.2 ± 5.5	0.12 – 10	0.9990	0.04	5.60	1.12 ± 0.08	0.93 ± 0.16
DB[a,e]P	99.5 ± 2.8	2.30 – 10	0.9940	0.70	2.81	9.08 ± 0.20	9.47 ± 0.51
DB[a,i]P	102.7 ± 3.2	0.47 – 10	0.9994	0.14	3.12	3.87 ± 0.17	3.64 ± 0.36
N[2,3-a]P	102.6 ± 4.1	0.43 – 10	0.9955	0.13	4.00	4.29 ± 0.45	3.97 ± 0.39
DB[a,h]P	102.2 ± 6.0	0.27 – 10	0.9992	0.09	5.87	2.50 ± 0.15	2.50 ± 0.27

W. B. Wilson, B. Alfarhani, A. F. T. Moore, C. Bison, S. A. Wise, A, D. Campiglia, Talanta, 148, 444, 2016.

Current Studies

- We are extending RPLC-LETRSS to the analysis of the remaining isomers with MM 302Da
- We are applying RPLC-LETRSS to the analysis of sediment samples from the Gulf of Mexico.





- ***Deepwater Horizon*** (DWH) was an ultra-deep water, offshore oil drilling rig owned by Transocean and leased to British Petroleum (BP) from 2001 until September 2013.

- On 20 April 2010, while drilling at the coast of Louisiana - Mississippi Canyon Block 252 or MC252 - an explosion on the rig caused by a blowout killed 11 crewmen and ignited a fireball visible from 40 miles (64 km) away.

- The resulting fire could not be extinguished and, on 22 April 2010, *Deepwater Horizon* sank, leaving the well gushing at the seabed and causing the largest oil spill in U.S. waters.

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