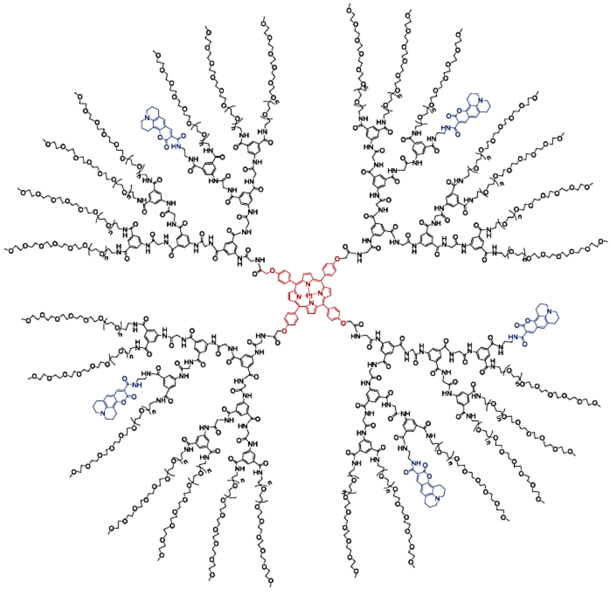
Lecture 3

Phosphorescent O_2 nano-probes

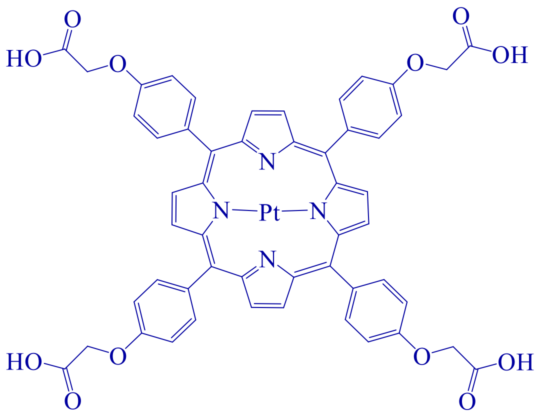


Outline

- The history and development of phosphorescent O2 probes
- Structure and sensing mechanism
- Synthetic approach (design, synthesis, purification, characterization)

A brief history
Early works

- 1990s decade
- Porphyrin complexes of Pd^{2+} and Pt^{2+}



- Interactions with other biomolecules in blood
- Bound to serum albumin

A brief history
Providing biological compatibility

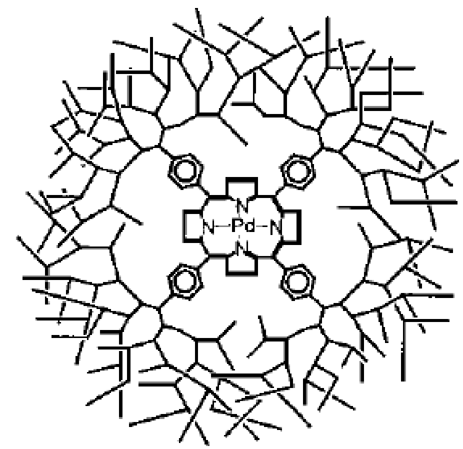
- 1999-2011

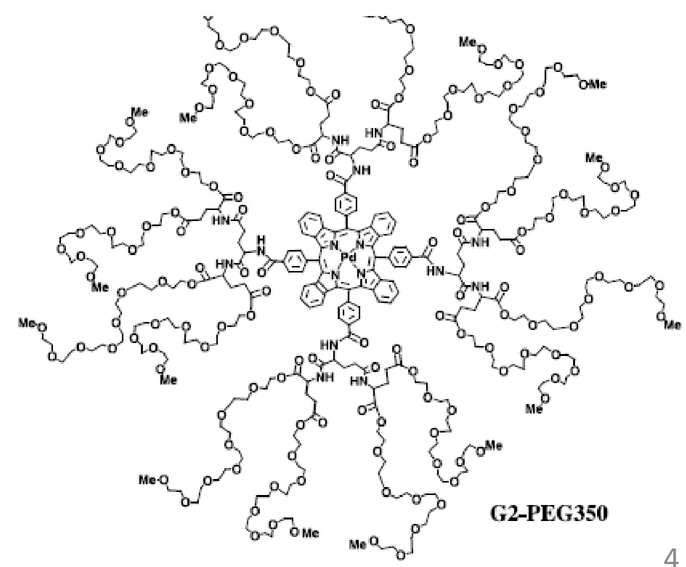
  Vinogradov et al. (1999) Chem. Eur. J. 5, 1338.

  Rietveld et al. (2003) Tetrahedron, 59, 3821.

  Esipova et al. (2011) Anal. Chem. 83, 8756.

- Dendritic protection (encapsulation)
  - Low toxicity
  - Low immunoreactivity
  - Neutrality (no sensitivity to pH, other molecules, etc)
  - Water solubility
  - Controlled size and molecular weight distribution
  - Fine-tuning of oxygen diffusion and quenching properties of the molecule sensitivity and dynamic range





G2-PEG350

A brief history
Providing biological compatibility

- Choice of dendrimer:
  - Stable (not degraded by enzymes, not targeted by the immune system)
  - In the case of partial decomposition, shows no toxicity
  - Possibility of functionalization of the outer layer (for fine-tune of the environment of the porphyrin)
  - Highly soluble in water
  - 3D conformation (determines the O2 diffusion and quenching properties)
  - Lack of aggregation in aqueous solutions
  - Ease of reactions with good yields

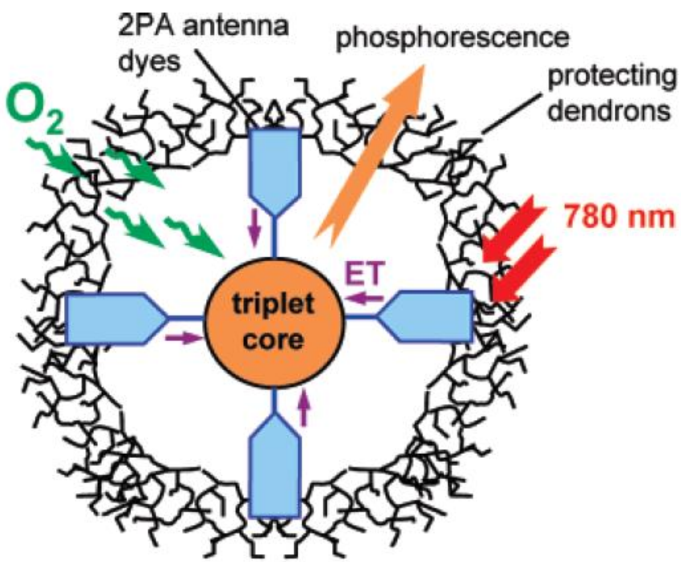
A brief history
Combination with two-photon microscopy

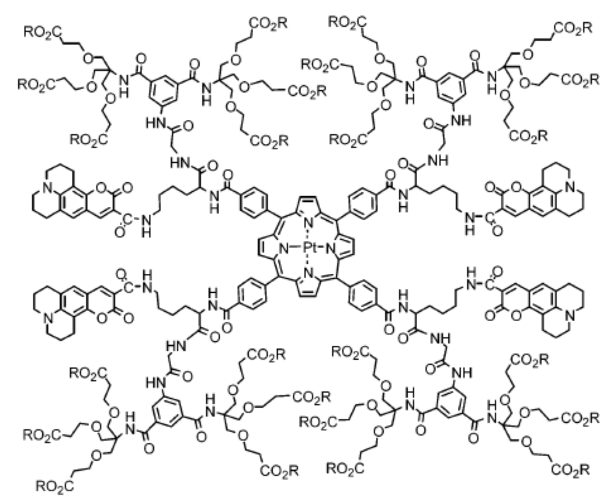
- 2005

  Brinas et al (2005) **J. Am. Chem. Soc.** 127, 11851.

- Extremely low 2Ph absorption of Pt and Pd porphyrin-based dyes

- Amplification of 2PA induced phosphorescence: 2P absorbing antenna and intramolecular energy transfer (ET)





12: R = ^{t}Bu
13: R = H

A brief history
Combination with two-photon microscopy

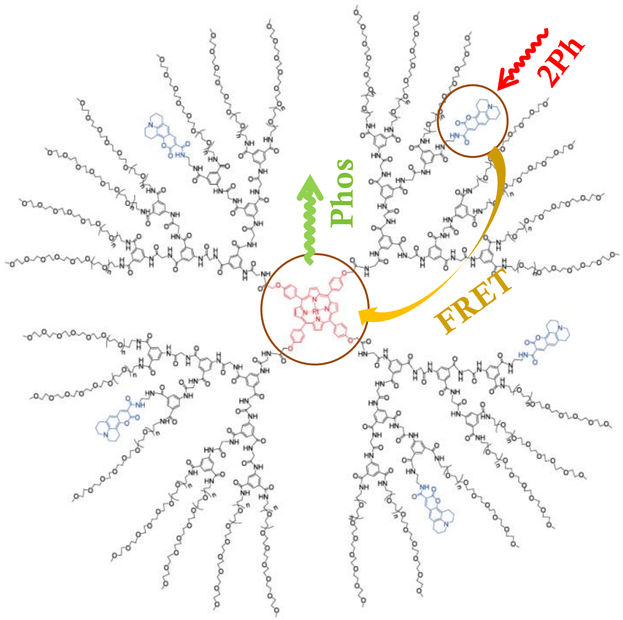
- 2Ph antenna maximal absorption in near infrared window (700-900 nm)
- Overlap between the fluorescence of antenna and absorption of porphyrin core
- Large amplification of the phosphorescent signal upon 2P excitation
- Acceptable phosphorescence quantum yield: Adjustment of 3D conformation, number of antenna chromophores , their separation, and their distance to core

A brief history
PtP-C343 dye

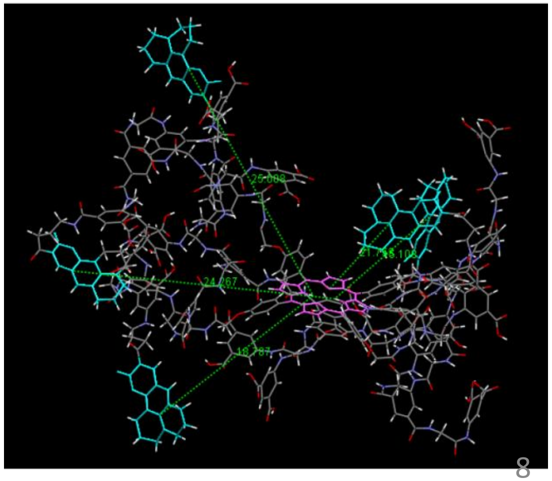
- **2008**

  Lebedev et al. (2008) *J. Porphyrins Phthalocyanines,* 12, 1261.

  Finikova et al. (2008) ChemPhysChem, 9, 1673.

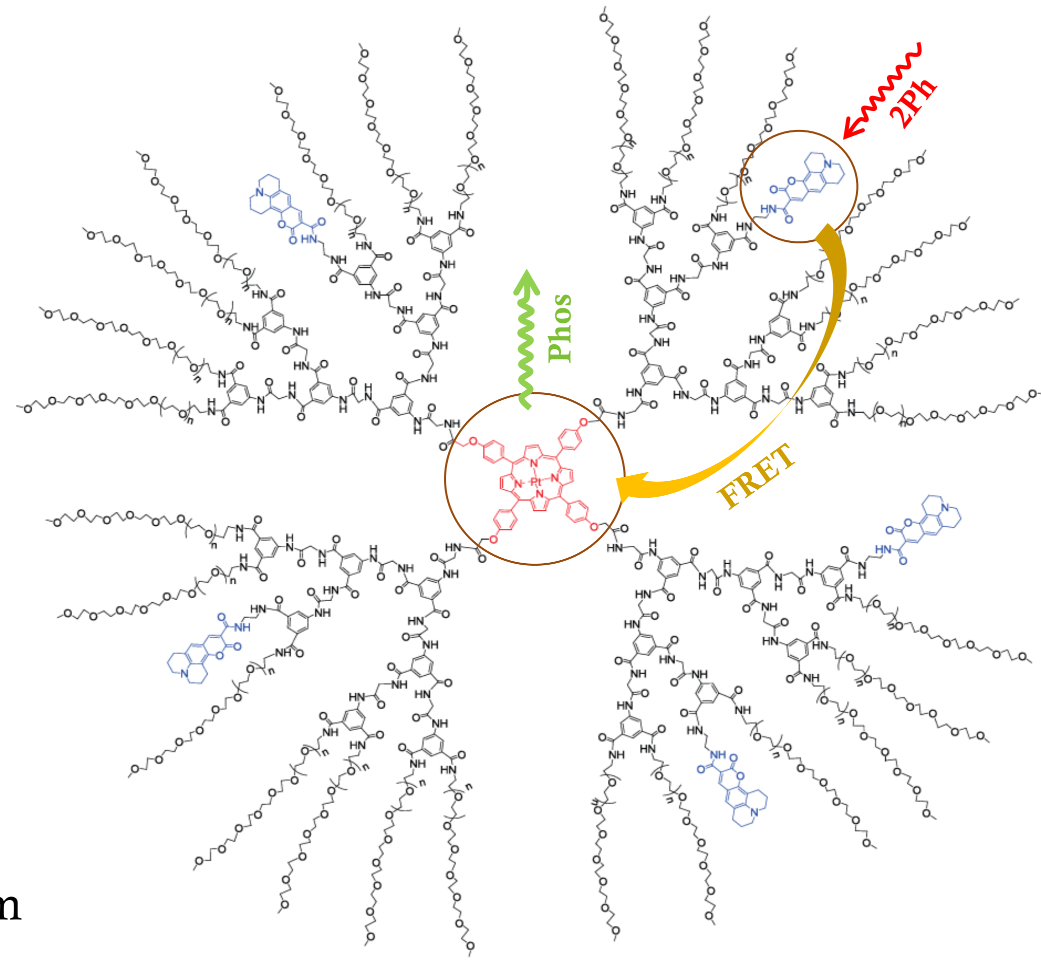


- The first practical 2P phosphorescent nanoprobe for in vivo oxygen imaging
- Tuning the distances between the antenna and the core
- Adjusting their redox potentials
- Prevention of unwanted electron transfer (intramolecular quenching of phosphorescence)



A brief history
PtP-C343 dye

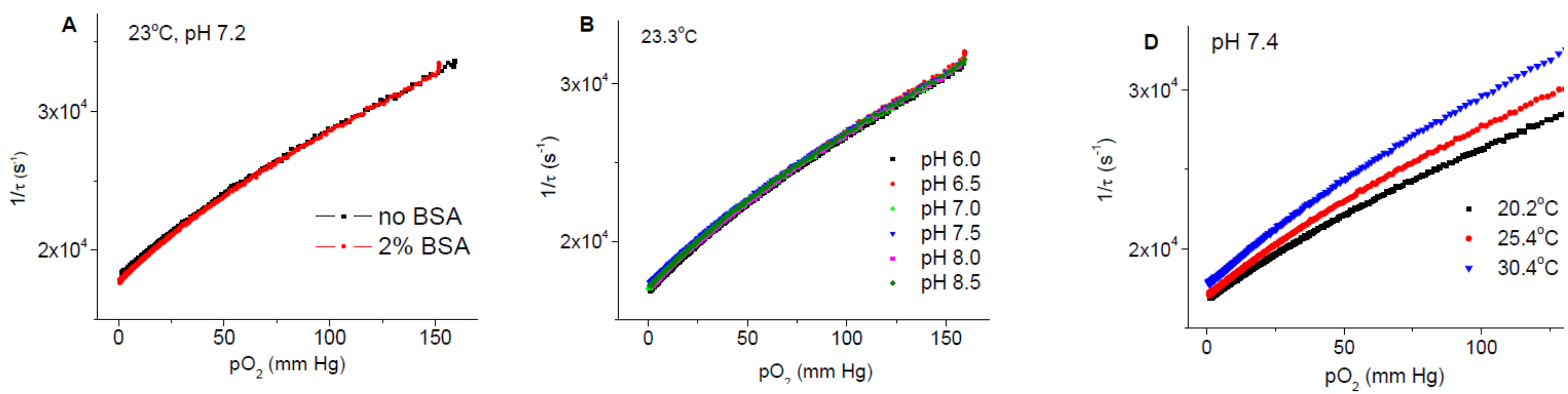
- Core: Pt-porphyrin
  - O2-sensitive
- Dendrimer:
  - Distance adjustment
  - Limiting the O2 diffusion
- 2Ph antennas: Coumarin 343
  - Enhanced 2Ph absorption
- PEG units (750-2000 Da)
  - Aqueous solubility
  - Nautrality



- τ_0~ 60 µs
- λ_{ex} (2Ph)=840 nm, λ_{em}=670 nm
- MW: 62,800, Size: ~ 5 nm
- Quantum yield: 10%

A brief history
PtP-C343 dye

- No effect on cell viability
- No interaction with other biomolecules
- Not sensitive to pH



A brief history
Enhanced phosphorescence quantum yield

- 2014

  Roussakis et al. (2014) Anal. Chem. 86, 5937.

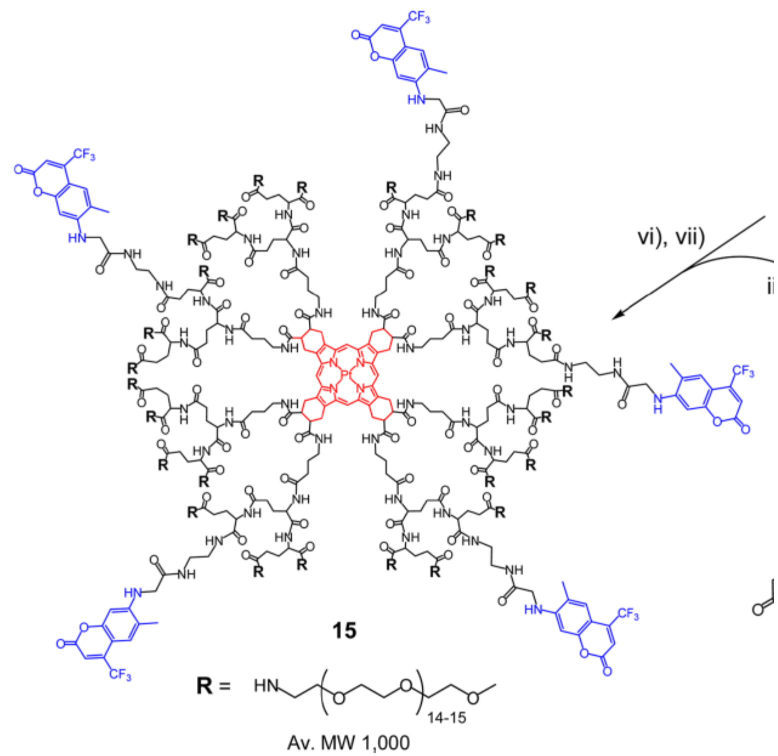
PtTCHP-C307 dye

Modified porphyrin core and antenna

Reduction of emissivity loss by further limitation of unwanted electron transfers

Significant increase in the phosphorescence quantum yield

Up to 6-fold higher signal output.



A brief history
Two-photon probes with no antenna

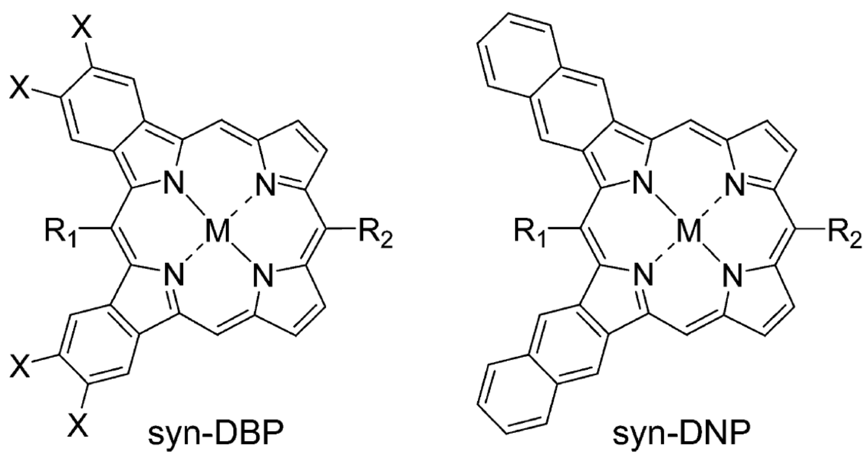
- 2014

  Esipova et al (2014) J. Org. Chem. 79, 8812.

Asymmetrically π-extended Pt and Pd porphyrins

More difficult to synthetize

Enhanced 2PA brightness and phosphorescence quantum yield

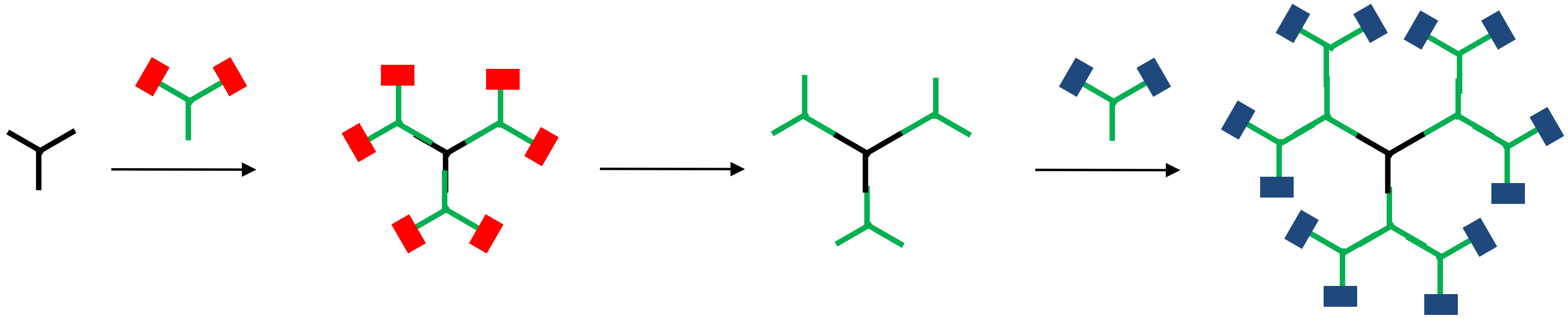


Synthetic approach
Design

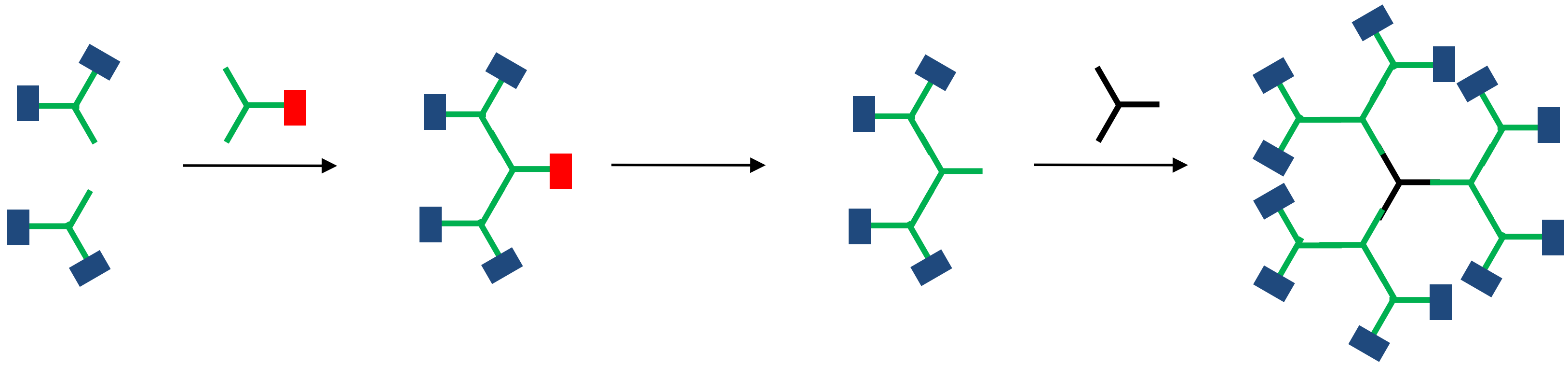
- Ease of reactions and purifications
- Good yield
- Stability of the dye and the intermediate compounds
- No toxicity, Solubility in water, No aggregation in aqueous solutions
  - Dendritic structure (structure, generation)
  - Encapsulation (number of PEG units, MW, functionalization)
- Appropriate photophysical and quenching properties
  - Dendritic structure (diffusion barrier, electron transfer)
  - 2Ph antenna – porphyrin core pairs (absorption and emission bands, redox potentials)
  - Control over the number of 2Ph antennas and their location
  - 3D conformation (antenna-antenna and antenna-porphyrin distances)

Synthetic approach
Design

- Divergent synthesis



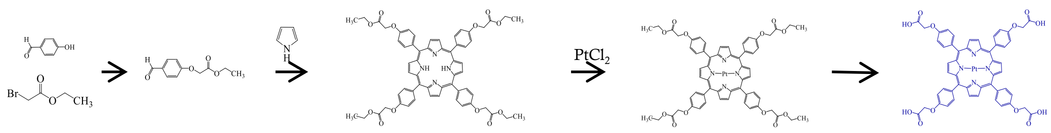
- Convergent synthesis



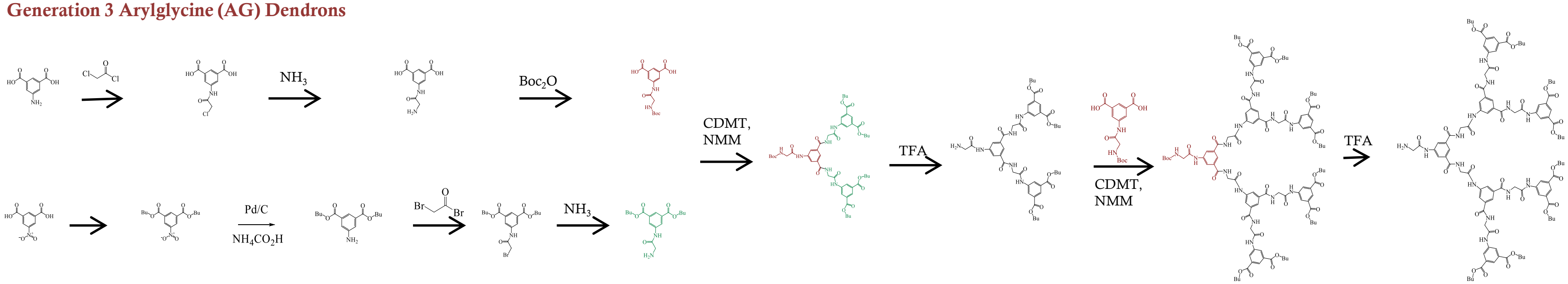
- Combined divergent/convergent approach

Synthetic approach
PtP-C343

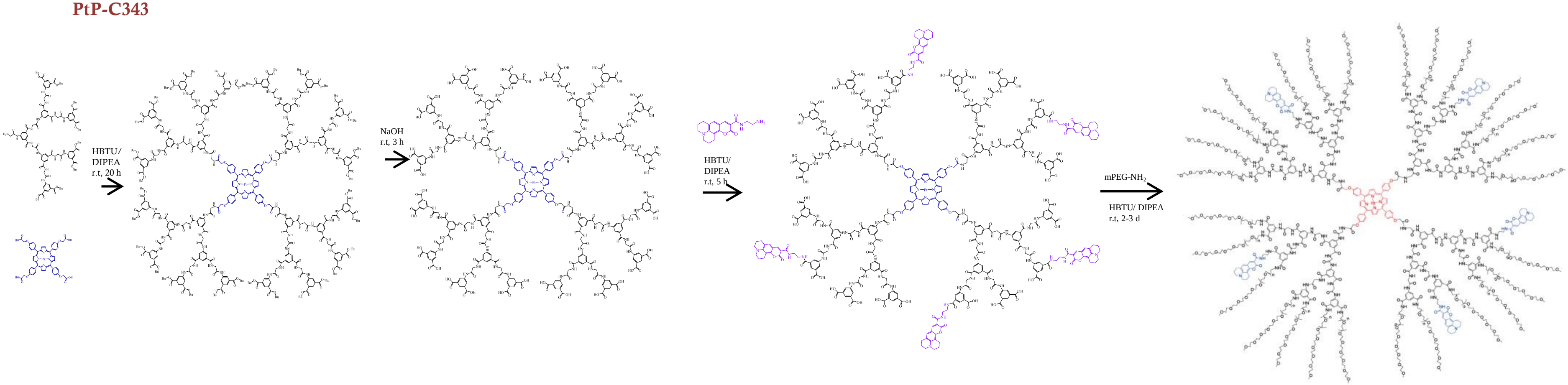
Pt-Porphyrin Core



Generation 3 Arylglycine (AG) Dendrons



PtP-C343

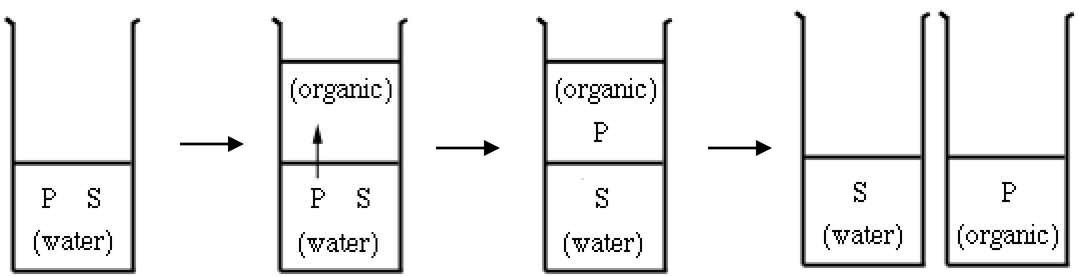


Synthetic approach
Purification

- Washing and extraction
- Filtration
- Precipitation and crystallization
- Chromatography
- Centrifugation

Purification
Washing and extraction

- Selective removal of one compound from a mixture using a solvent
- Washing: solid-liquid
- Extraction: liquid-liquid
- Solvent and mixture must be immiscible
- The compound must be more soluble in the solvent than in the mixture



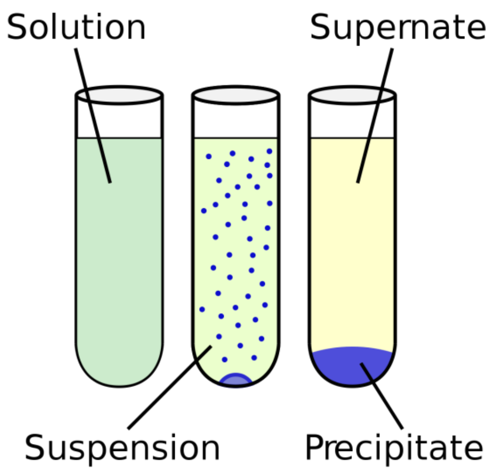
Separatory funnel

Purification
Filtration

- Solid-liquid systems
- Size exclusion
- Vacuum
- Filter papers

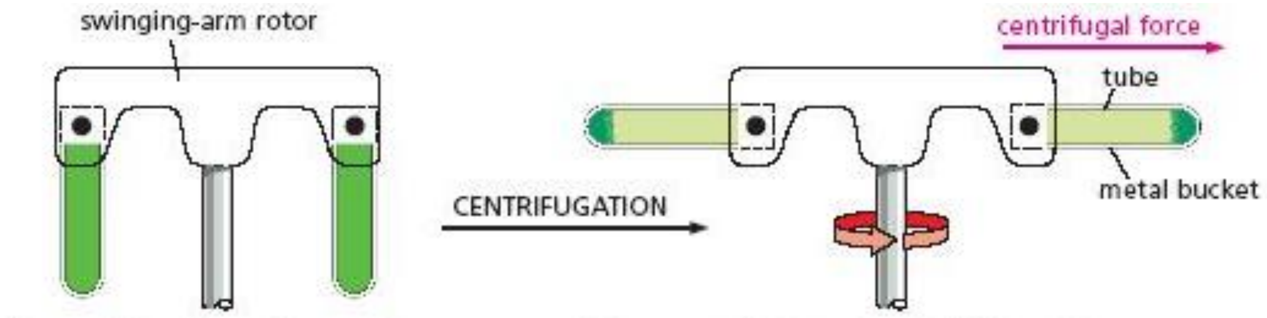
Purification
Precipitation and crystallization

- Creation of a solid from a solution
- Precipitation: rapid formation of solid
- Crystallization: slow formation of a crystal network
- Solubility change
  - Cooling
  - Addition of another solvent
  - Acidification



Purification
Centrifugation

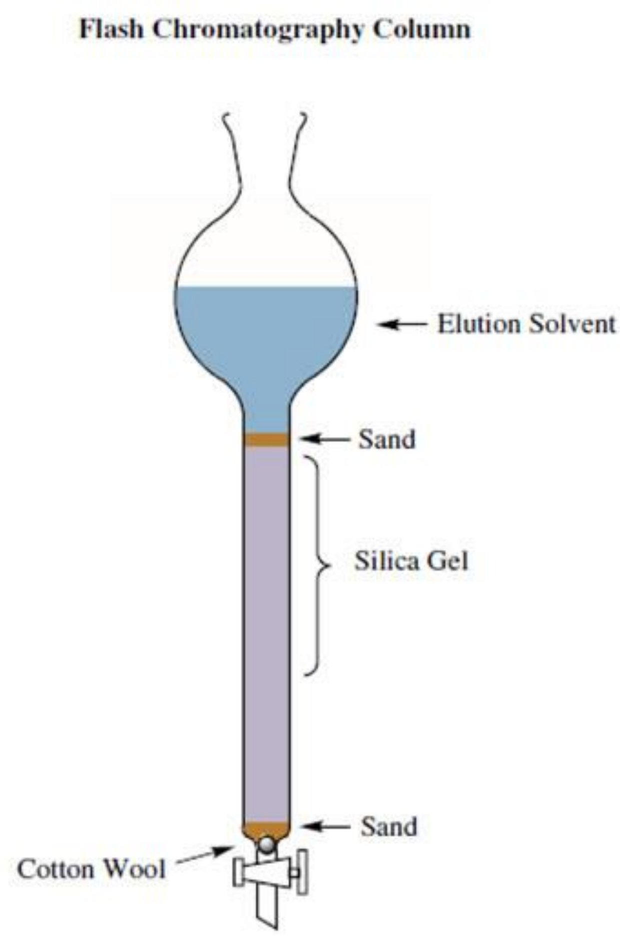
- To accelerate the precipitation process
- Suspensions; emulsions
- Centrifuge tubes
- 3000 – 10000 rpm



Purification
Chromatography

- Column chromatography
  - Mobile phase
  - Stationary phase
- The various compounds travel at different speeds, causing them to separate.

- Flash chromatography
- Size-exclusion chromatography



Purification
Chromatography

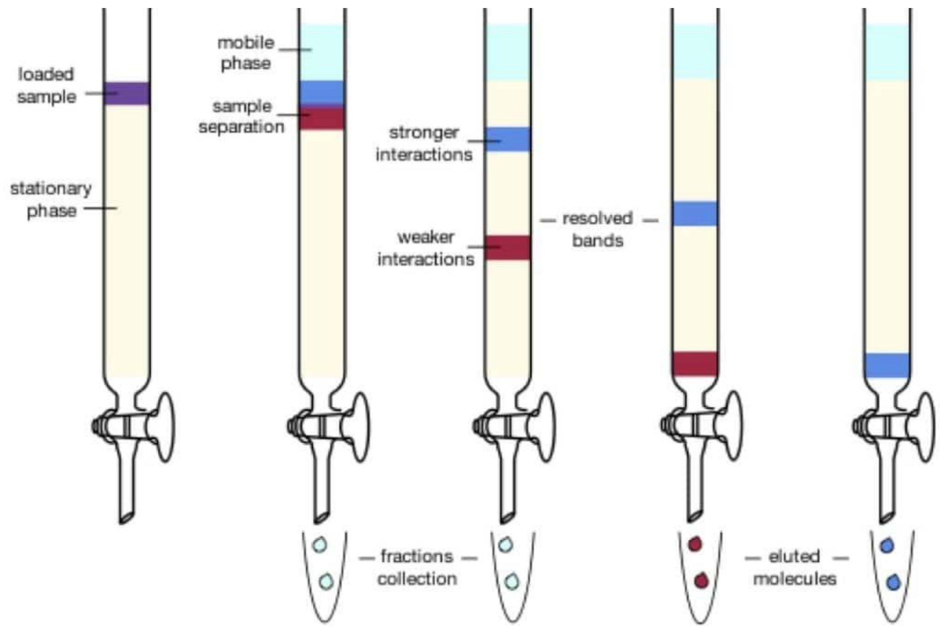
- **Flash chromatography**

- Polar stationary phase (silica gel (SiO_2); alumina (Al_2O_3))
- Non-polar or weakly polar mobile phase

- Separation based on component polarity
- More polar compound ➔ stronger interaction ➔ slower movement

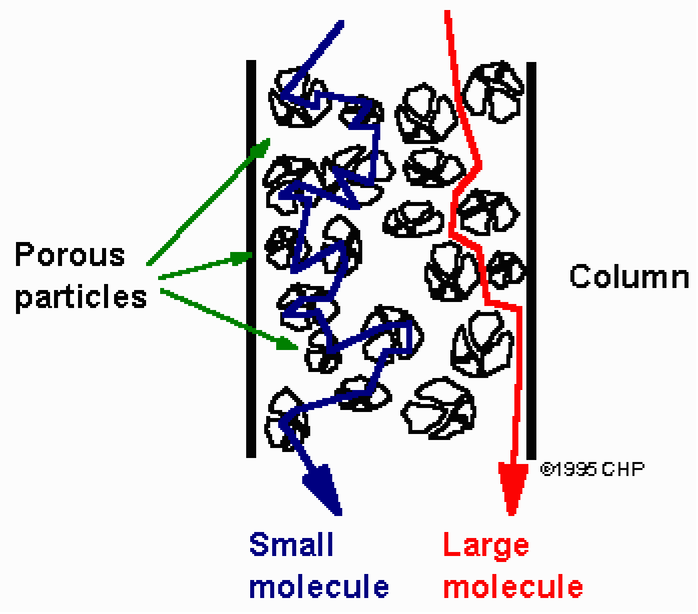
- Mobile phase may be a mixture, allowing fine-tuning the component separation

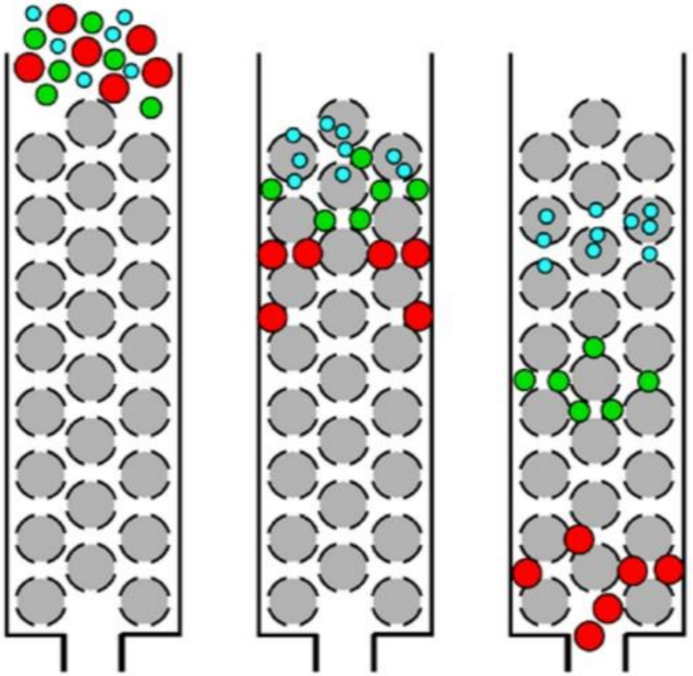
- Positive pressure (N_2)



Purification
Chromatography

- **Size-exclusion chromatography (gel permeation chromatography, GPC)**
- Stationary phase with fine porous structure (cross-linked dextran gel)
- Based on component size



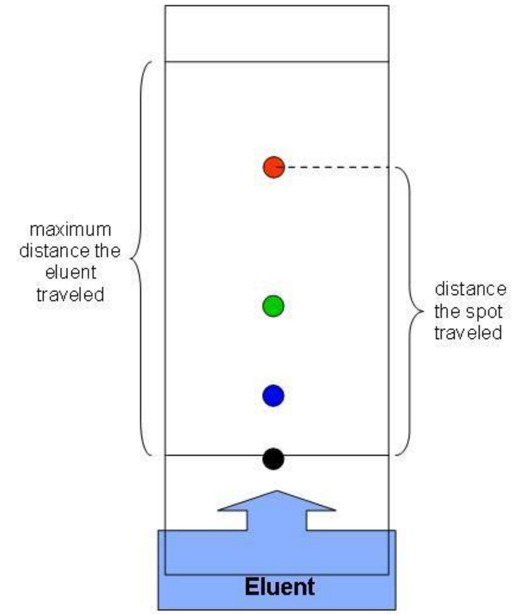


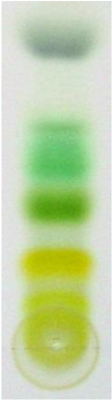
Synthetic approach
Characterization

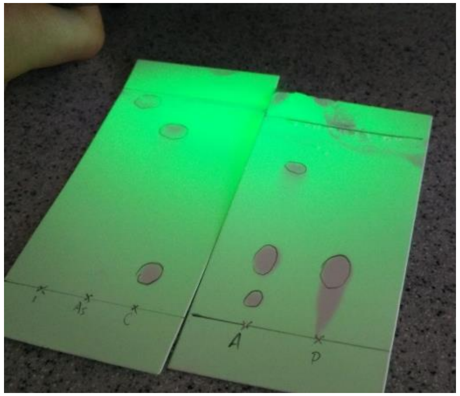
- Thin layer chromatography (TLC)
- Nuclear magnetic resonance (NMR) spectroscopy
- Mass spectroscopy (MS)
- UV-Vis spectroscopy
- Gel permeation chromatography (GPC)
- Fourier-transform infrared spectroscopy (FTIR)

Characterization
TLC

- Similar to column chromatography
- A sheet of glass, plastic, or aluminum foil, coated with a thin layer of adsorbent material (silica gel or alumina)
- Capillary action
- Visualization of spots, usually by projecting ultraviolet light
- Ideal for monitoring the reaction completion







Characterization
NMR

- Nuclear magnetic resonance (NMR): absorption and re-emission of electromagnetic radiation by NMR active nuclei (such as ^{1}H or ^{13}C) in a magnetic field

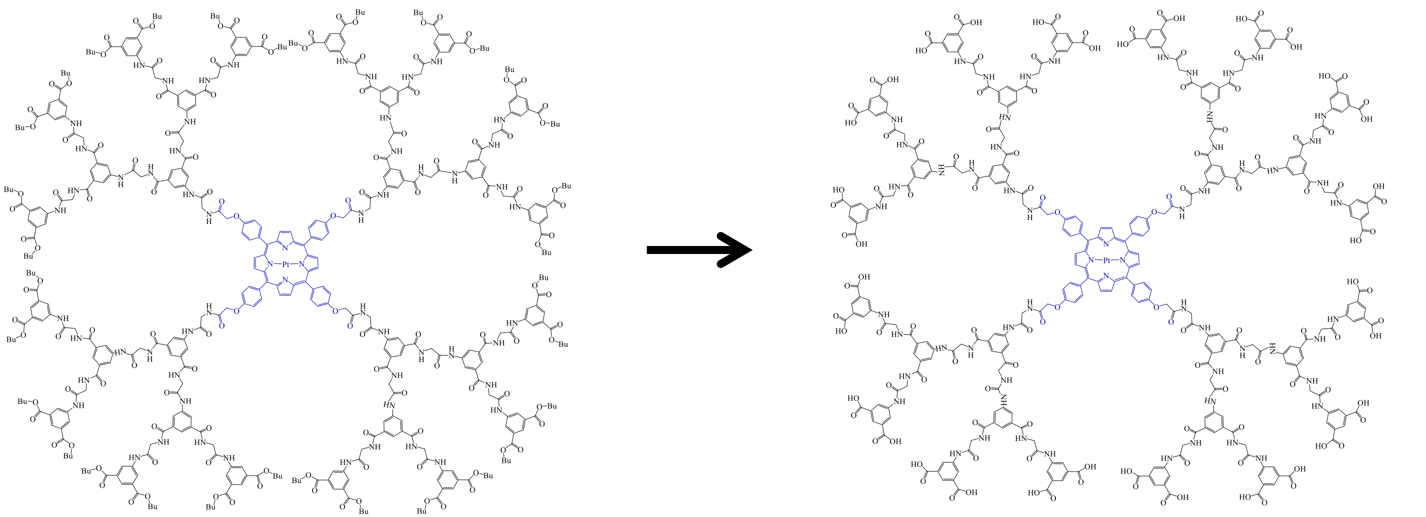
- At a specific resonance frequency (chemical shift) which depends on the magnetic properties of the atoms

- Diagnostic of the structure of the molecule

- Different functional groups

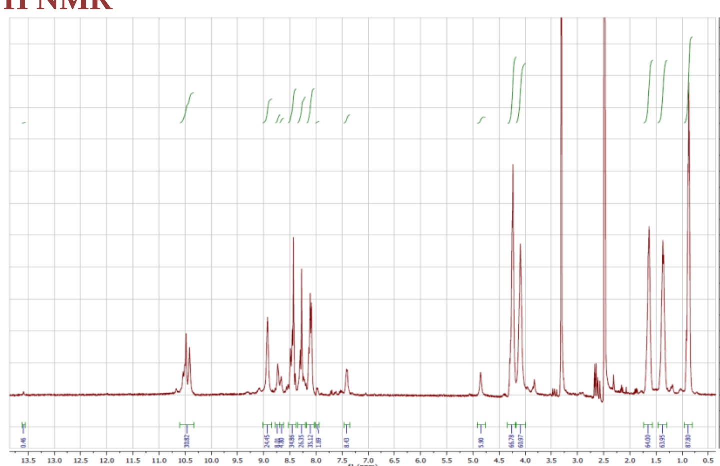
- Identical functional groups with differing neighboring substituents

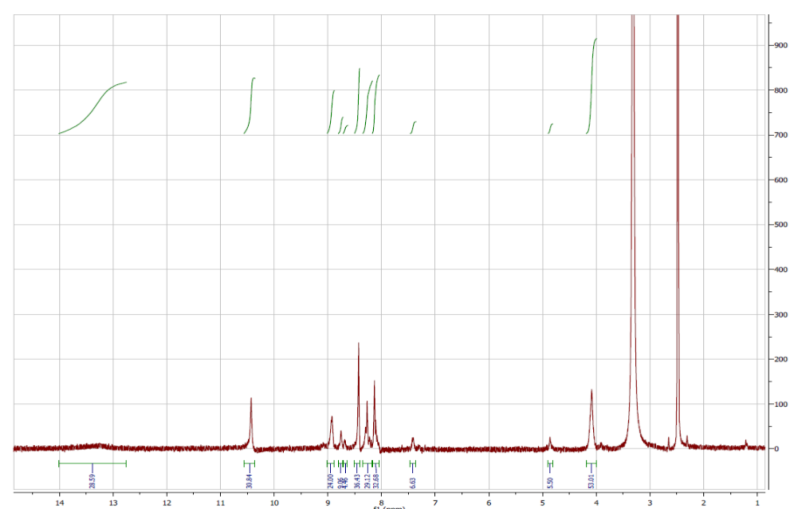
Characterization
NMR



PtP-(AG^{3}OBu)$_4$ → **PtP-(AG^{3}OH)$_4$**

H NMR





Characterization
Mass spectroscopy

Ionization

Acceleration, subject to an electric and/or magnetic field

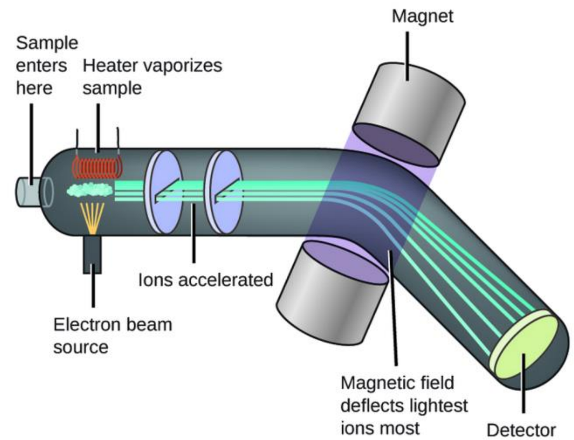
The speed and direction of charged particles movement depends on their mass-to-charge ratio

Ion detection (electron multiplier)

Characterization
Mass spectroscopy

Electrospray ionization (ESI)

Ion production using an electrospray

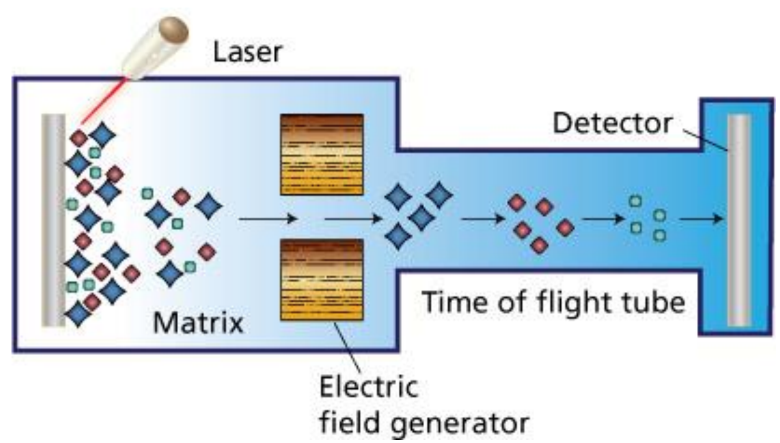


Matrix-assisted laser desorption/ionization (MALDI)

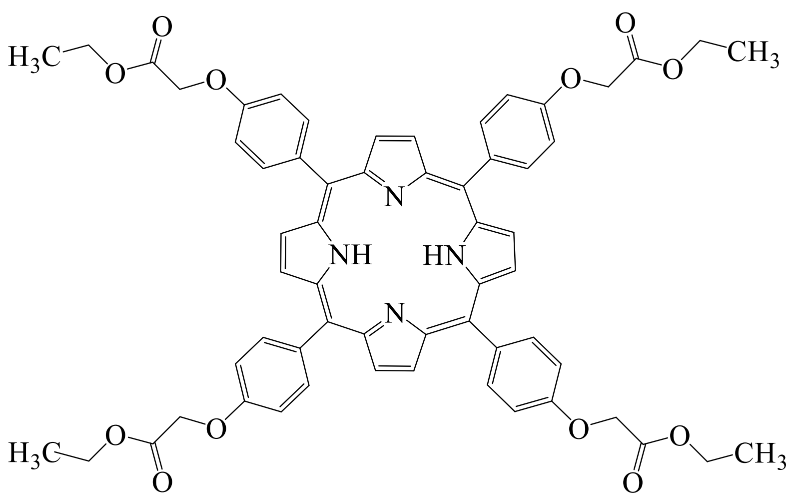
Ion creation using a laser energy absorbing matrix

Mixing the sample with a suitable matrix material

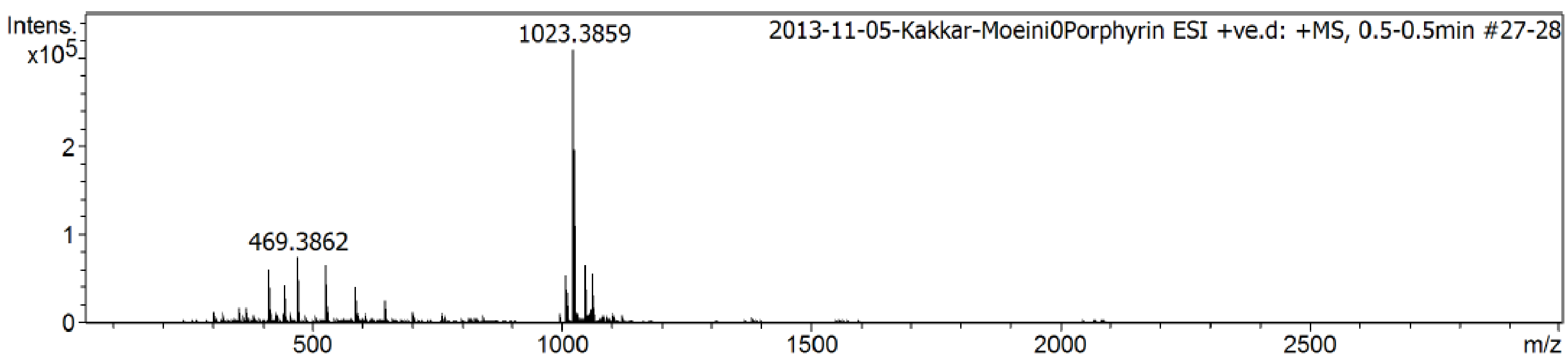
Pulsed laser irradiation



Characterization
Mass spectroscopy

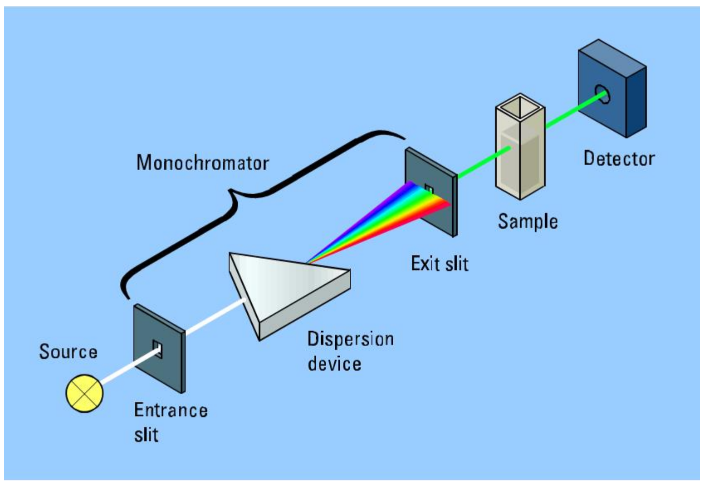


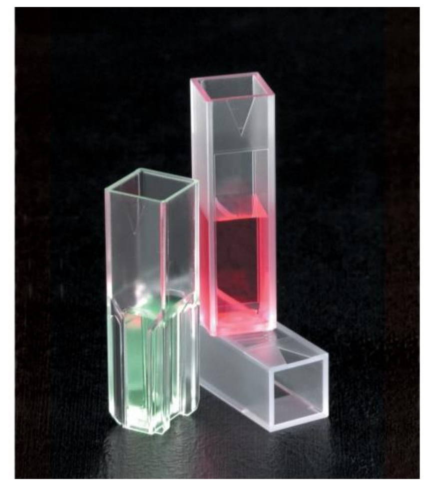
Calculated MW: 1023.1



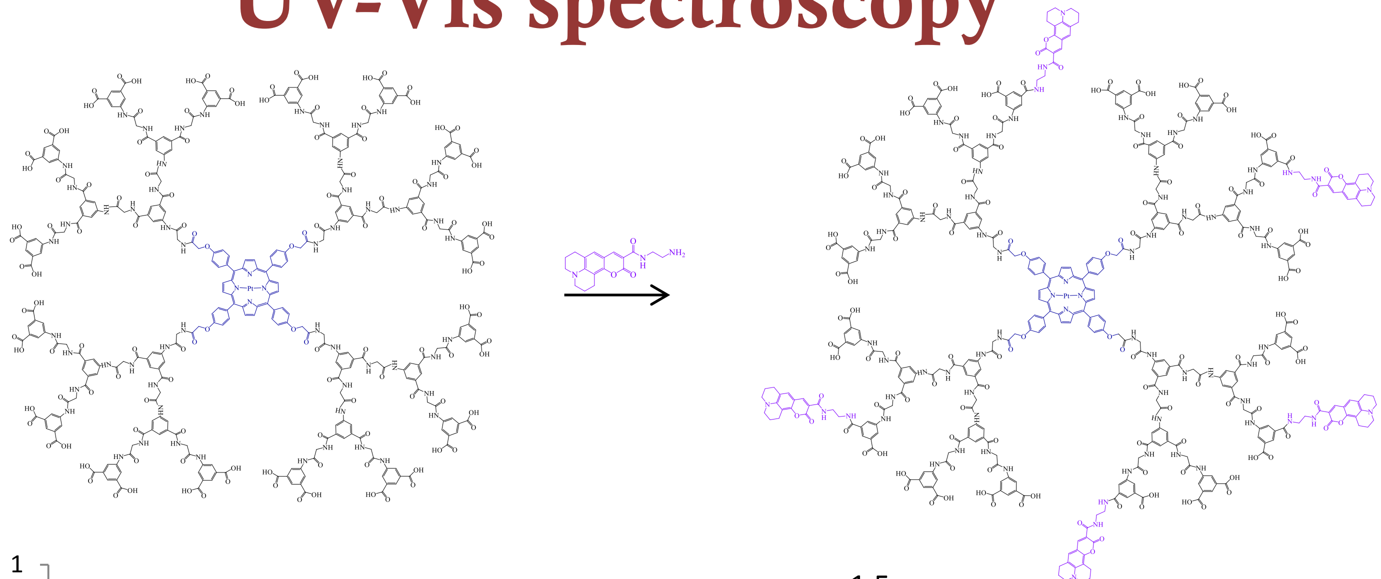
Characterization
UV-Vis spectroscopy

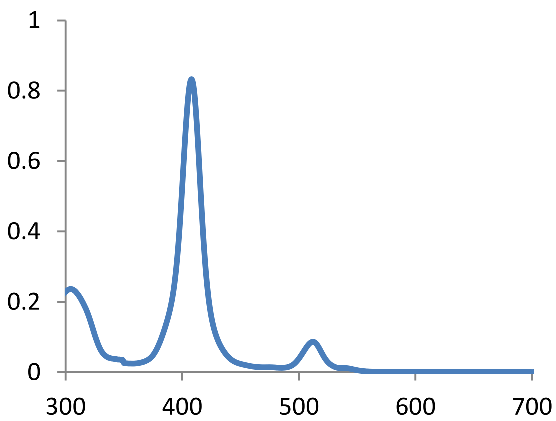
- Absorption spectroscopy in the ultraviolet-visible spectral region

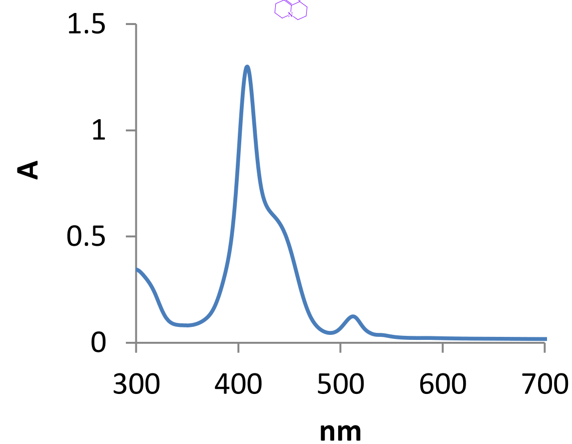




Characterization
UV-Vis spectroscopy

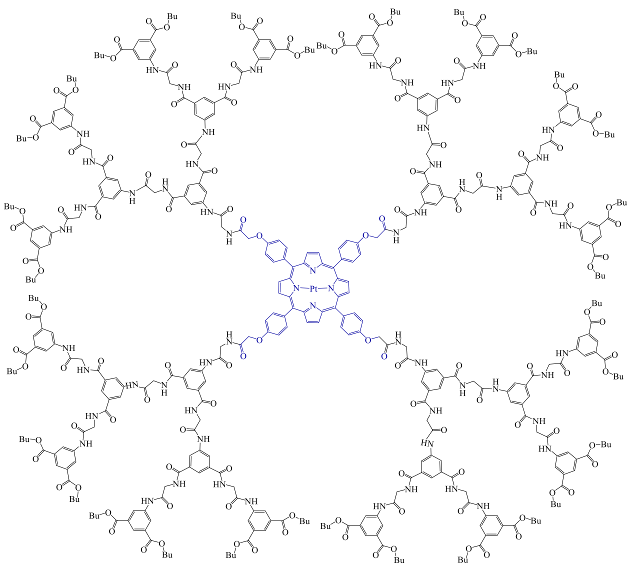


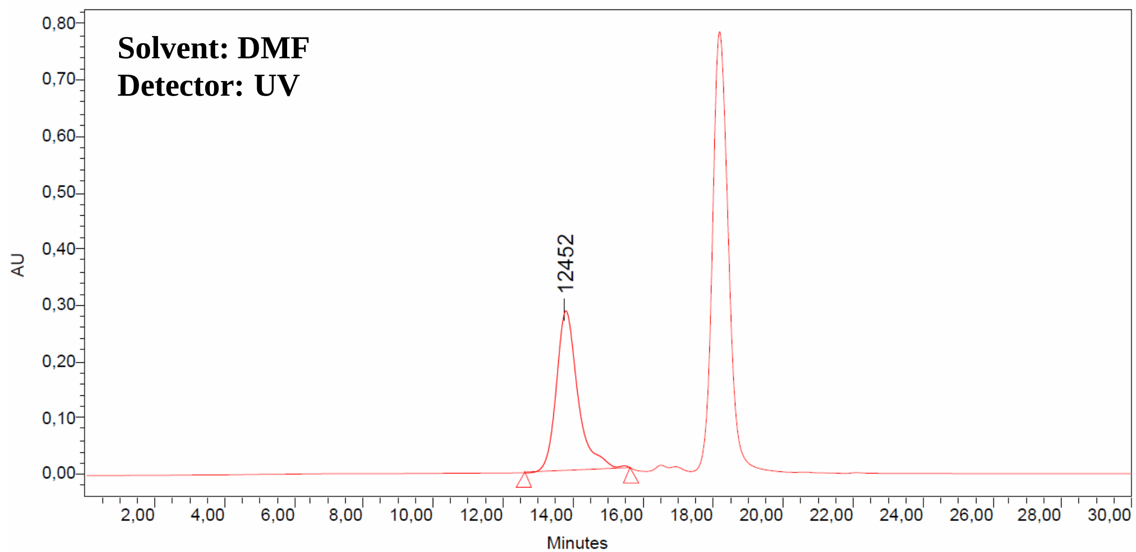




Calculated dye/dendrimer molar ratio: ~ 5
Expected ratio: 4-5

Characterization
GPC





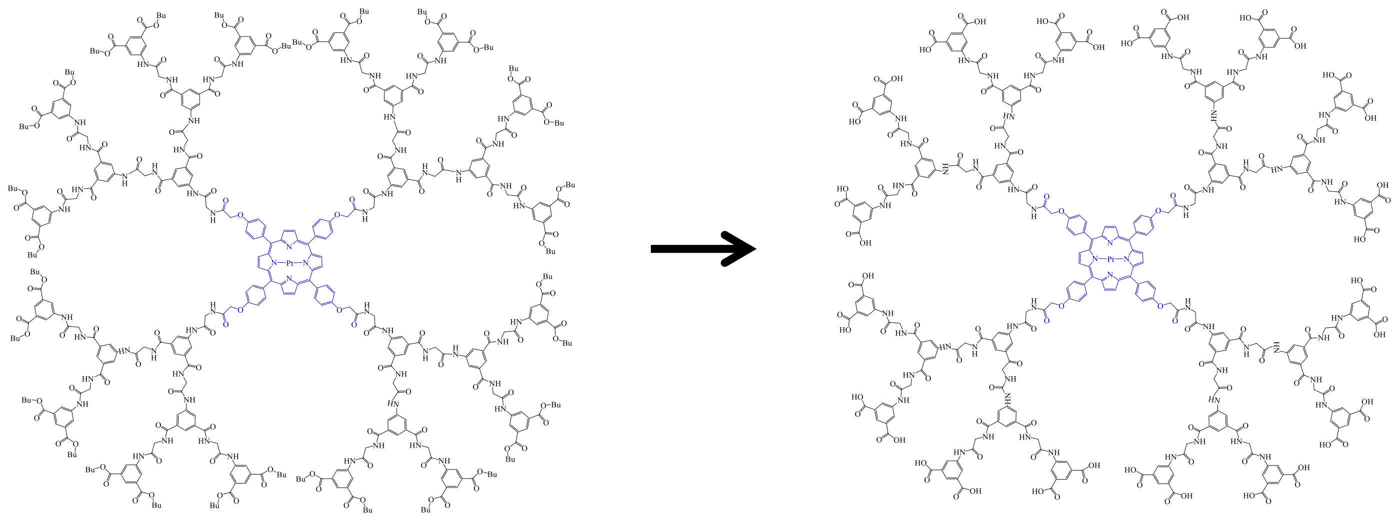
GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		9329	12021		12452	14171	16170	1,288644		

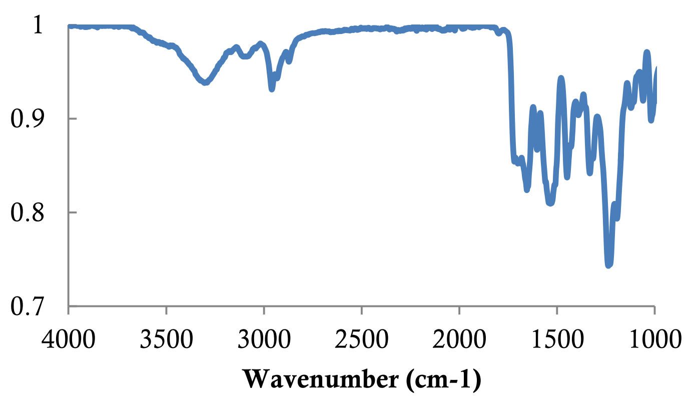
Characterization
FTIR

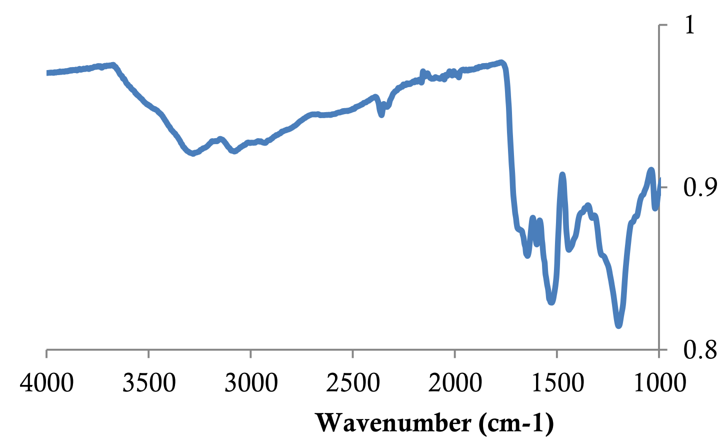
- Infrared absorption spectrum
- Solid state

Characterization
FTIR



PtP-(AG^{3}OBu)$_4$ PtP-(AG^{3}OH)$_4$





Forster-type resonance energy transfer (FRET)

- Energy transfer between two light-sensitive molecules (chromophores) through nonradiative dipole–dipole coupling
- Over distances between 10 and 100 Angstrom
- Extremely sensitive to small changes in distance (inversely proportional to the sixth power of the distance between donor and acceptor)