Leibniz-Institut für Polymerforschung Dresden

Polymers in Solution

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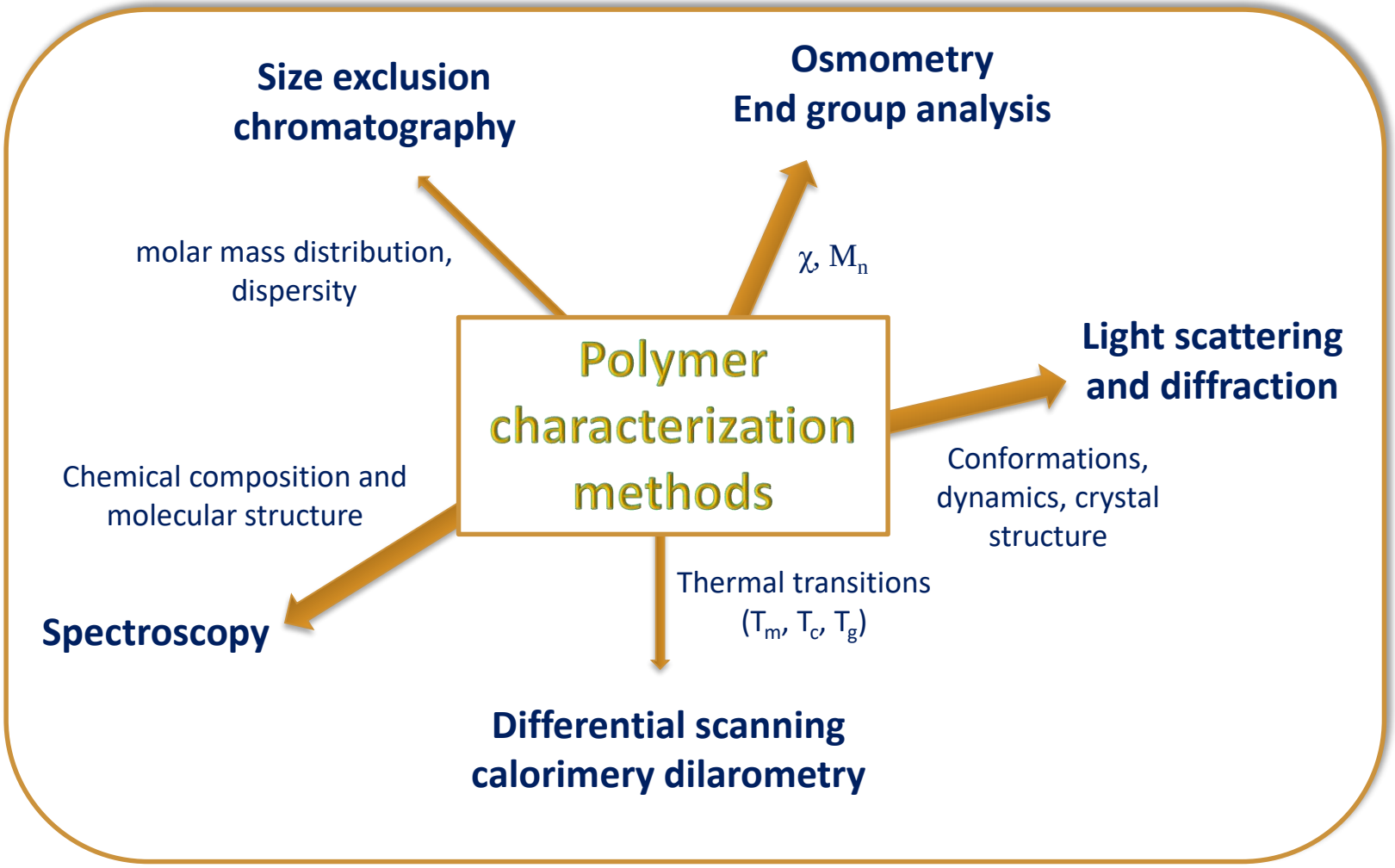
DRESDEN concept

Leibniz Gemeinschaft

5. Molar mass determination

1. Overview



Aggregation (in solution): Dynamic light scattering , field-flow fractionation, small-angle X-ray, fluorencesce spectroscopy, UV-VIS spectroscopy

1. Overview

Molar Mass in Polymers

Number average molar weight

$$M_n = \frac{\sum_i c_i}{\sum_i (c_i/Mi)} = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

Weight average molar weight

$$M_w = \frac{\sum_i (ciM_i)}{\sum_i c_i}$$

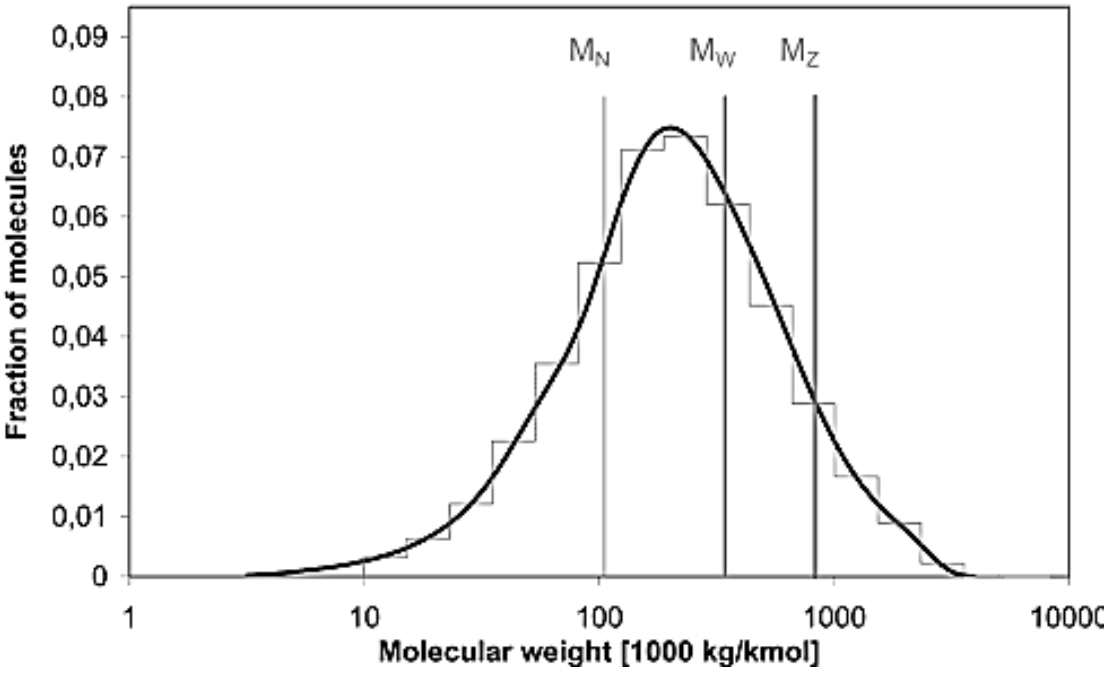
z-average molecular weight

$$M_z = \frac{\sum_i (ciM_i^2)}{\sum (c_i M_i)} = \frac{\sum_i z_i M_i}{\sum z_i}$$

MMD (polydispersity) is with $\mathbf{M_w/M_n}$

For **monodisperse** samples $\mathbf{M_w/M_n = 1}$

Polydisperse polymers have $\mathbf{M_w/M_n > 1}$



2. Methods for determining the molar mass of macromolecules

Method	Molar mass average	Molar mass range
Absolut method		
Ebulliometry, cryoscopy	M_n	$M < 5 \times 10^3$
Membrane osmometry	M_n	$10^4 < M > 10^6$
Isothermal distillation	M_n	$M > 5 \times 10^4$
Sedimentation velocity	M_n, M_w, M_z	$M > 10^2$
Equilibrium sedimentation	M_w, M_z	$M > 10^2$
Vapor pressure osmosis	M_n	$M < 2 \times 10^4$
Static Light Scattering	M_w	$M > 5 \times 10^2$
Turbidity measurements	M_w	$M > 5 \times 10^2$
Small-angle X-ray scattering	M_w	$M > 5 \times 10^2$
Small-angle neutron scattering	M_w	$M > 5 \times 10^2$
Dynamic Light Scattering	M_w	$M > 5 \times 10^2$
Mass spectroscopy- MALDI-TOF	M_n, M_w, M_z	$M > 5 \times 10$
Equivalent method		
End-group analysis- (titration, NMR, IR)	M_n	$M < 5 \times 10^4$
Relative method		
Dilute solution viscometry	M_η	$M > 10^2$
Gel Permeation chromatography	M_n, M_w, M_z	$M < 10^7$
Supercritical fluid chromatography	M_n, M_w, M_z	$M < 10^7$
Field-flow fractionation	M_n, M_w, M_z	$M > 10^3$

2. Methods for determining the molar mass of macromolecules

Characterizing polymer structure with small-angle neutron scattering: A Tutorial

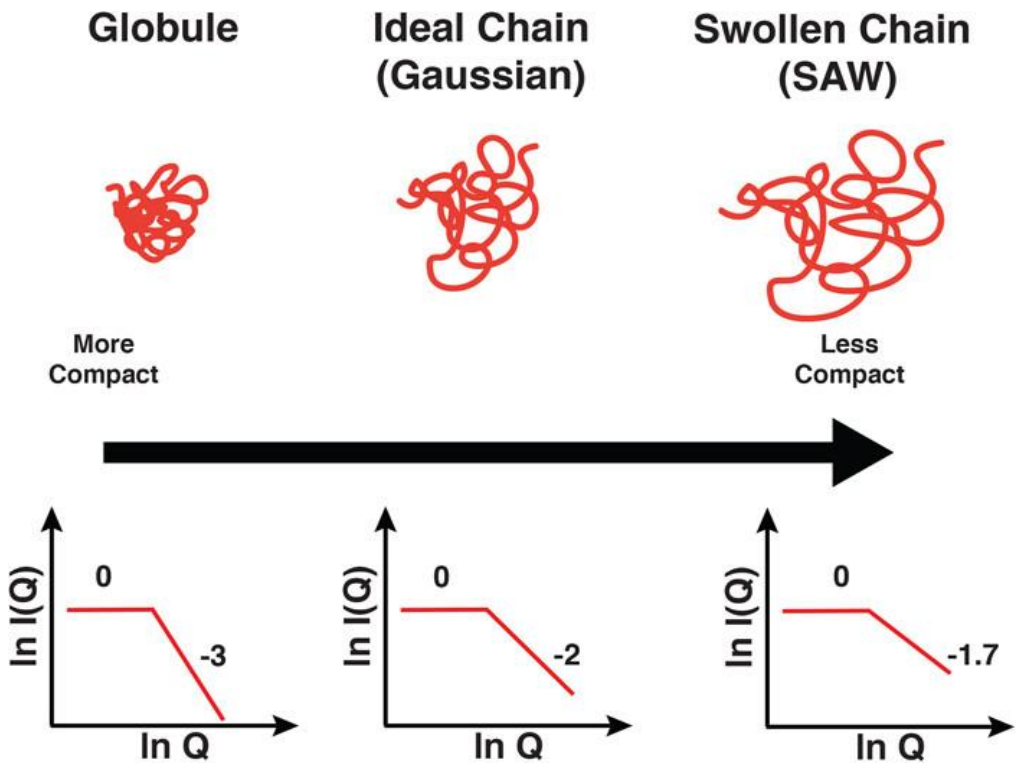


FIG. 5. Illustration of polymers with (left to right) globular, ideal, and swollen conformations. The corresponding Porod plots and slopes are shown below each.

Published in: Yuan Wei; Michael J. A. Hore; *Journal of Applied Physics* **129,** 171101 (2021)
DOI: 10.1063/5.0045841

3. Colligative properties and M_n

Colligative properties of solutions are properties that depend upon the concentration of solute molecules or ions, but not upon the identity of the solute. **Colligative properties include vapor pressure lowering, boiling point elevation, freezing point depression, and osmotic pressure.**

Cryoscopy

$$\left(\frac{\Delta T_f}{C}\right)_{C=0} = \frac{RT^2}{\rho \Delta H_f \overline{M}_n} + A_2 C$$

ΔT_f : freezing-point depression,
C : the concentration in grams per cubic centimeter
R : gas constant
T : freezing point
ΔH_f: the latent heats of fusion
A2 : second virial coefficient

Ebulliometry

$$\left(\frac{\Delta T_b}{C}\right)_{C=0} = \frac{RT^2}{\rho \Delta H_v \overline{M}_n} + A_2 C$$

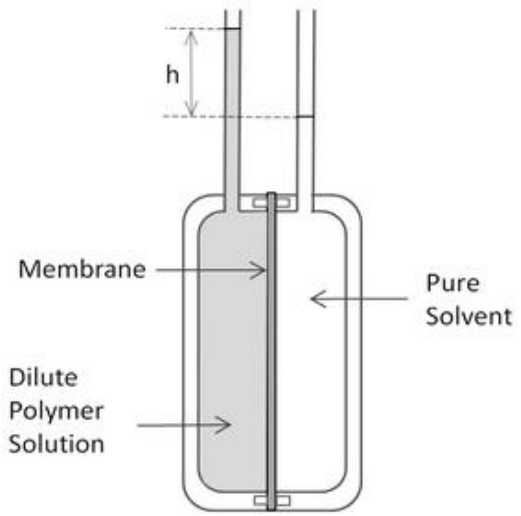
ΔT_b : boiling point elevation
ΔH_v : the latent heats of vaporization

M_n for low molar mass (≤ 10000 g/mol)

3. Colligative properties and M_n

Membrane osmometry METHODS BASED ON COLLIGATIVE PROPERTIES

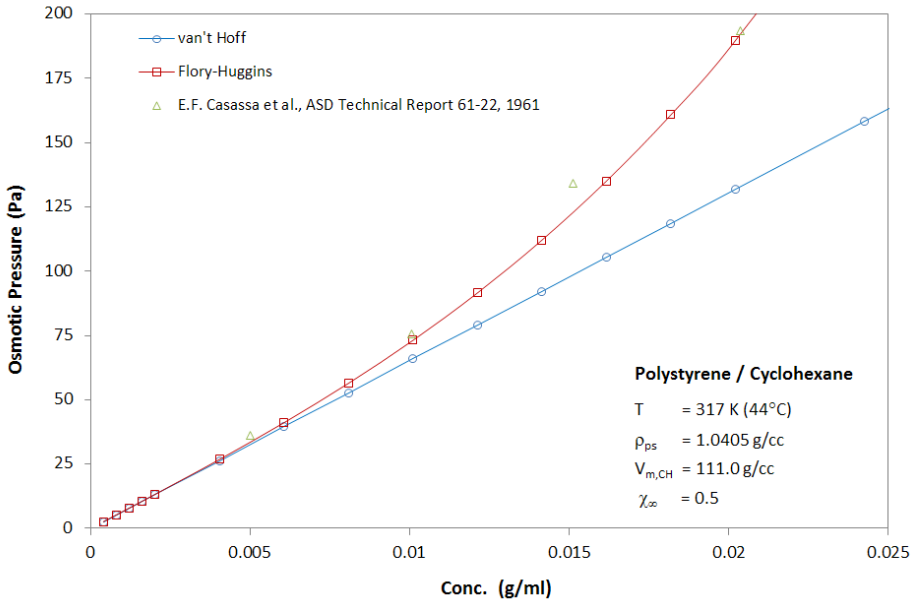
SIMPLE MEMBRANE OSMOMETER

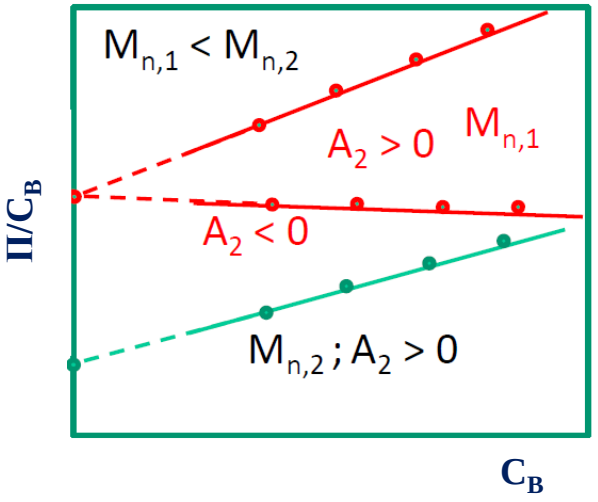


$10^4 < M_n > 10^6$

$$\frac{\pi}{c_B} = RT\left(\frac{1}{M_n} + A_2 c_B\right)$$

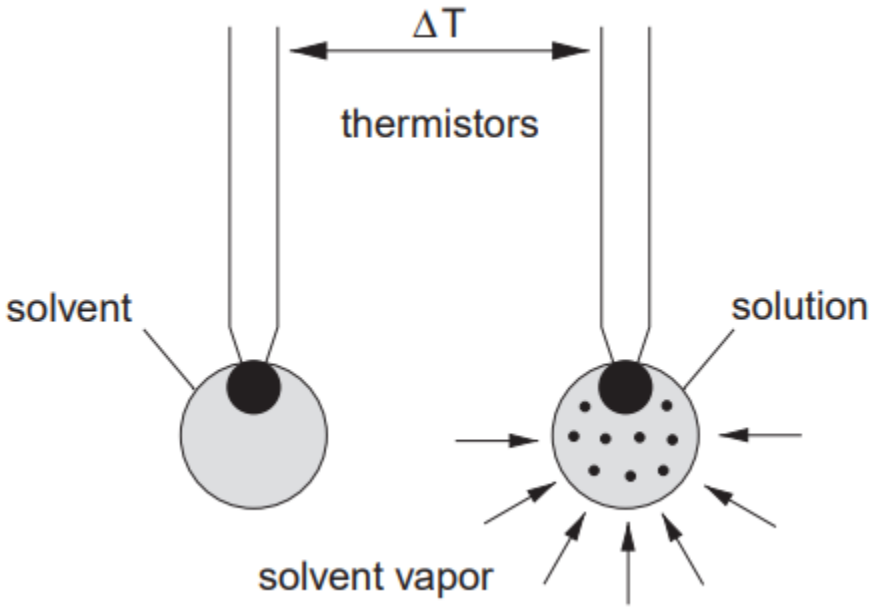
Determination of M_n (interception) and A_2 (slope)





3. Colligative properties and M_n

Vapour pressure osmometry METHODS BASED ON COLLIGATIVE PROPERTIES



$$\frac{p_0 - p}{p_0} = V_s^m \frac{c}{\langle M \rangle_n}$$

$$\frac{\Delta p}{p} = \frac{\Delta T}{T} \frac{\Delta H_{vap} p}{RT} = x_p = V_s^m \frac{c}{\langle M \rangle_n}$$

M_n for low molar mass (≤ 20000 g/mol)

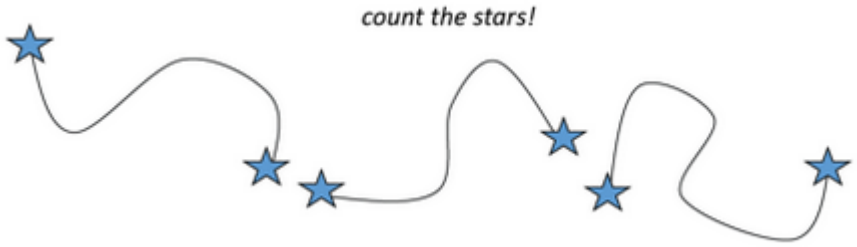
4. Equivalent method

End group analysis

$M_n \leq 50000$ g/mol

- Molecular weight limitation up to 50000 g/mol (the concentration of end groups has to be sufficient to get an accurate measurement)

- You have to know how many end groups there are per molecule (to find molar mass), OR you know the molar mass, and want to know number of end groups per molecule. **Limitation for branched polymers**



- End-group must have detectable species

a. Vinyl polymer: -CH=CH_2

b. Ester Polymer: -COOH, -OH

c. Amide and urethane polymer: -NH_2 , -NCO

d. Radioactive isotopes or UV, IR, NMR detectable functional groups

4. Equivalent method

How to Determine Hyaluronic Acid Molecular Weight Using Gel Electrophoresis

Hyaluronic Acid, is a natural non-sulfated glycosaminoglycan produced in many organs and tissues. It was discovered in the 1930s and was originally thought to have no physiological function except serving as a lubrication “space filler” between joints. With additional research, hyaluronic acid is now appreciated as an important part of the extracellular matrix. Indeed, it has critical roles in many cell signaling events such as proliferation, inflammation, wound healing, and fertilization.

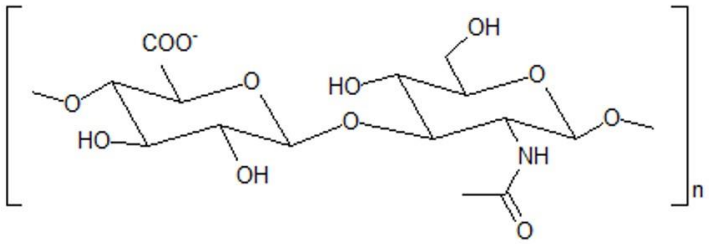


Figure 1. Repeating disaccharide units of Hyaluronic Acid

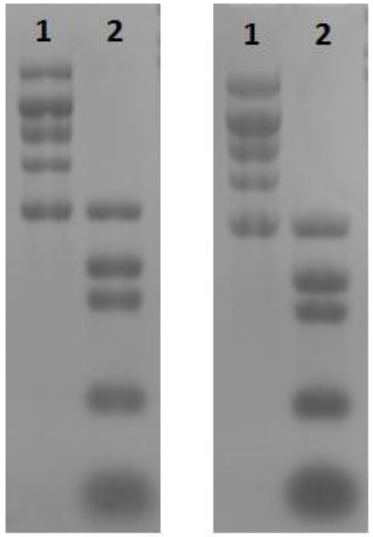


Figure 2. Source of Agarose

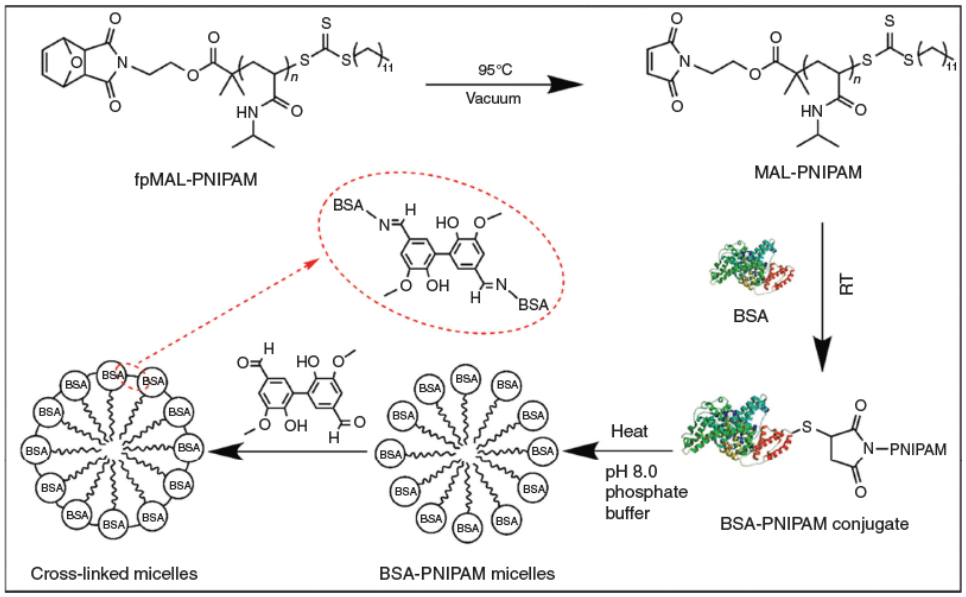
Select-HA™ Ladders were run on 1% agarose gel prepared using 2 different agarose source. Both agarose gels were run using same conditions.

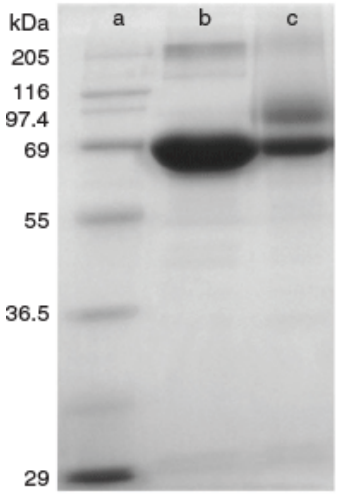
Agarose gel prepared using Source #1 resulted with more defined HA bands than agarose gel prepared using Source #2.

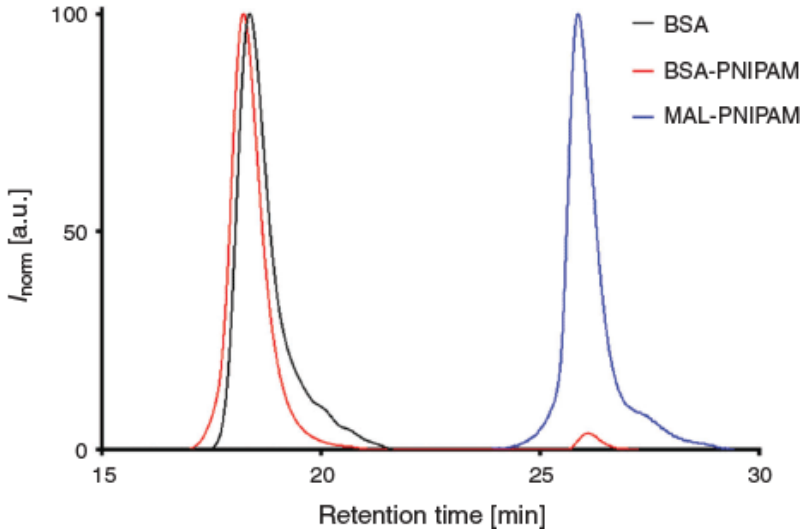
Lane 1 – 5 µL Select-HA™ HiLadder
Lane 2 – 5 µL Select-HA™ LoLadder

4. Equivalent method

Gel Electrophoresis





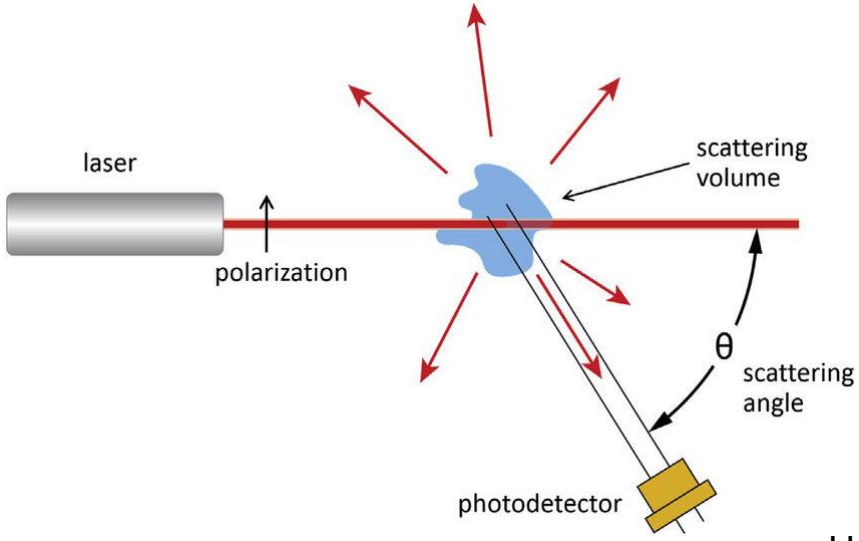


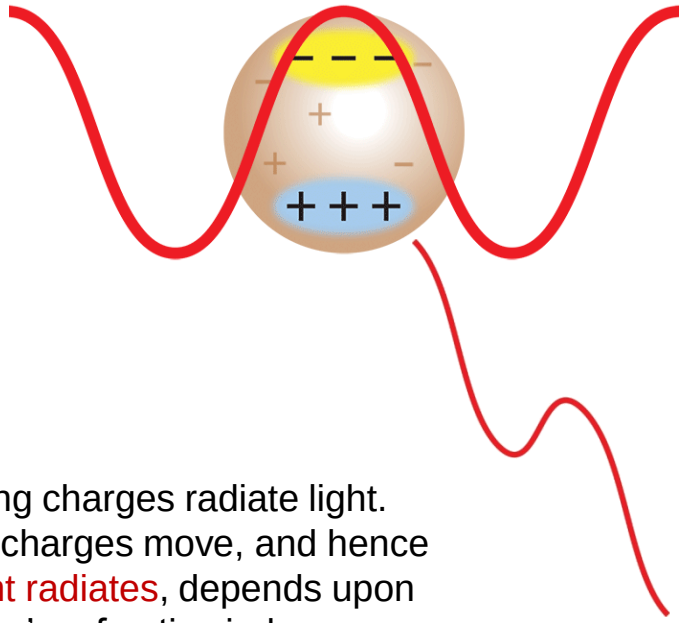
Li Zihao, Jiang Yanyan, Wüst Kilian, Callari Manuela, Stenzel Martina H. (2020) Crosslinking of Self-Assembled Protein–Polymer Conjugates with Divanillin. *Australian Journal of Chemistry* **73**, 1034-1041.

5. Absolut method

Static light scattering

Why can we use it to measure molar masses?





The oscillating charges radiate light. How much the charges move, and hence how much light radiates, depends upon the matter's refractive index.

The intensity of scattered light is **directly proportional to the molar mass**.

The angular dependency of scattered light is proportional to the size (radius).

5. Absolut method

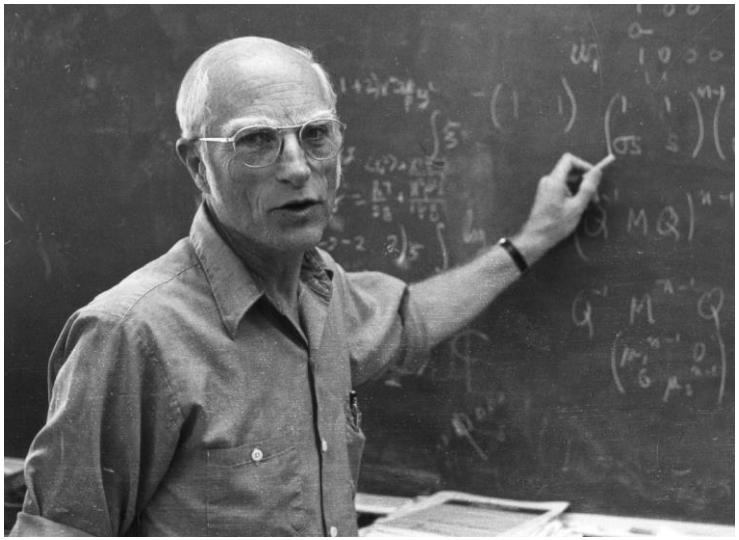
Static light scattering

From Rayleigh Ratio to molar mass

Zimm Equation, J. Chem. Phys. 16, 1093-1099 (1948).

$$\frac{K^* c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c$$

$K^* = 4\pi^2 (dn/dc)^2 n_0^2 N_A^{-1} \lambda_0^{-4}$



The amount of scattered light at scattering angle 0 is directly proportional to the product of **molar mass** (g/mol) and concentration (g/ml).

5. Absolut method

MALDI-TOF

The **Matrix Assisted Laser Desoption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF-MS)** is a fast and sensitive absolute method for determining both the num-ber average and weight average molar masses. It has a special status in the analysis of polymers of biological origin. In an ideal case, molar masses of <300,000 g/mol can be measured with an accuracy of ±0.01%

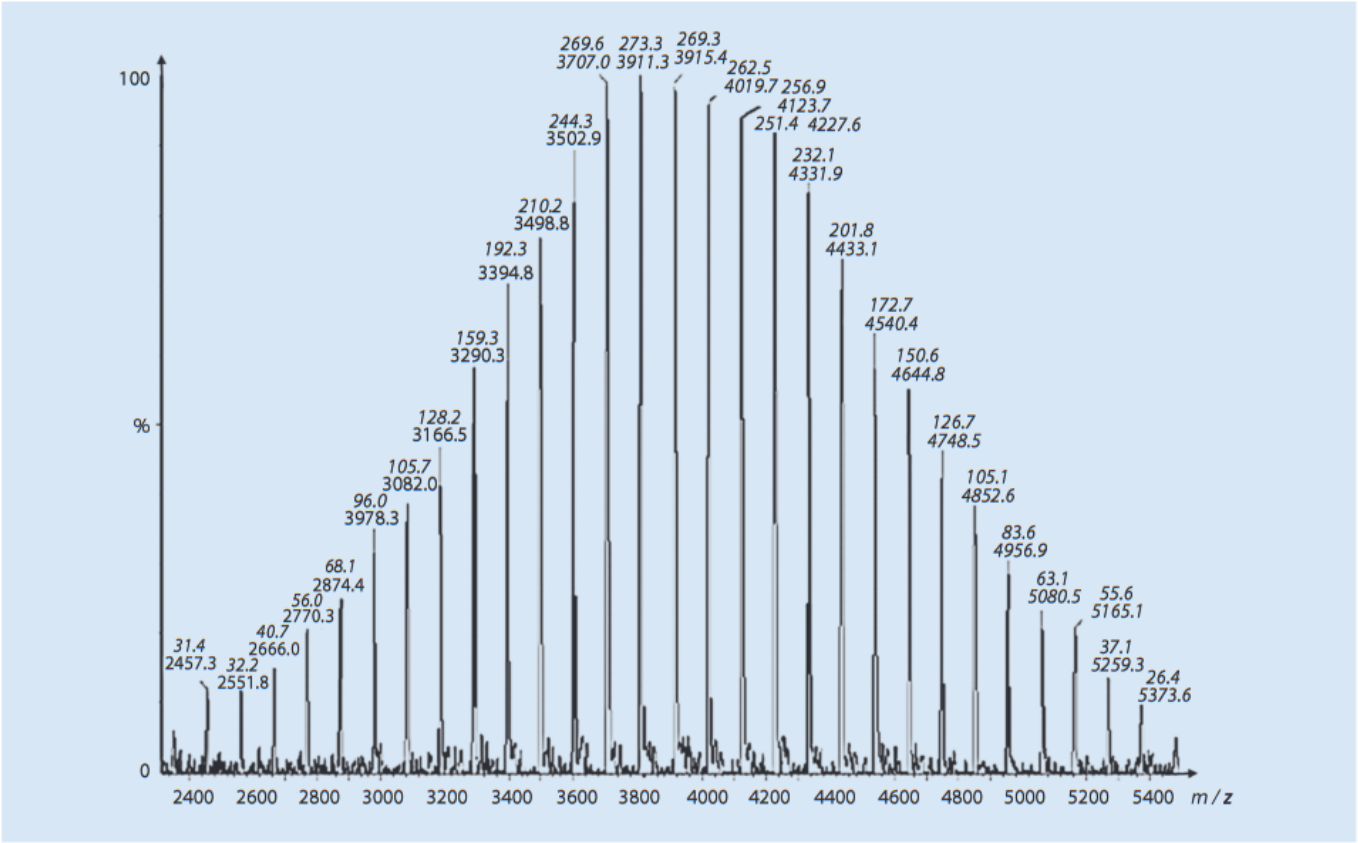


Fig. 3.16 MALDI-TOF-MS-spectrum of a polystyrene. The relative abundance (normalized to the most abundant peak) is plotted against the mass/charge ratio (*m/z*). The *numbers* above the peaks are the relative abundance and the corresponding *m/z* value

5. Absolut method

Ultracentrifuge

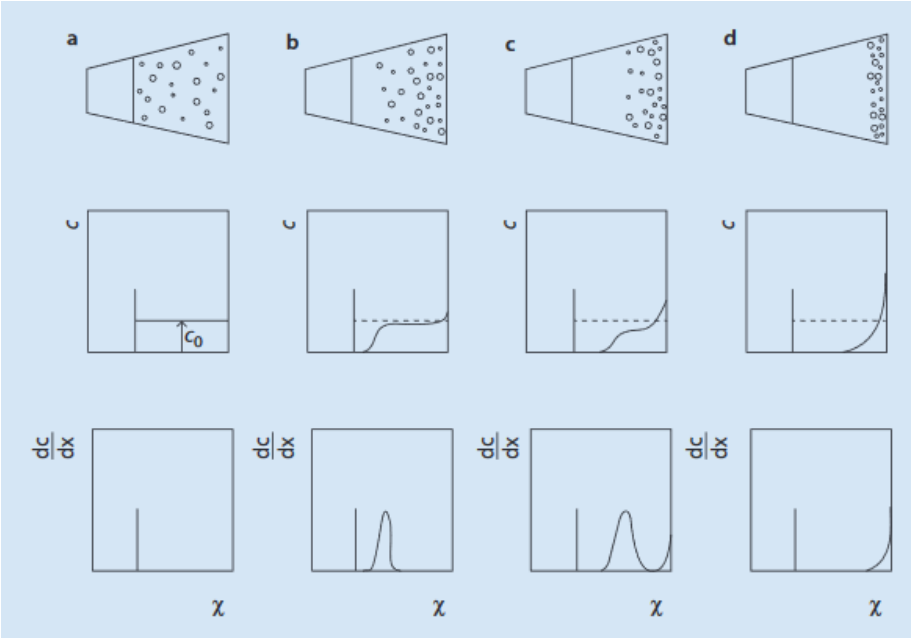
The ultracentrifuge (UC) is a centrifuge which rotates at very high speeds and was origi-nally developed by Svedberg for his research on inorganic and organic colloids (Svedberg and Pedersen 1940).

Three types of experiment are possible, which allow conclusions about **the shape, conformational changes, and size distribution of dispersed particles or dissolved macromolecules**.

(1) Analysis of Sedimentation Velocity

(2) Measurement at Thermodynamic Equilibrium

(3) Sedimentation Equilibrium in a Density Gradient



6. Relative method

Dilute solution viscometry

A. IUPAC suggested the terminology of solution viscosities as following.

Relative viscosity : $\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o}$

- η : solution viscosity
- η_o: solvent viscosity
- t : flow time of solution
- t_o: flow time of solvent

Specific viscosity : $\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \frac{t - t_o}{t_o} = \eta_{rel} - 1$

Reduced viscosity : $\eta_{rel} = \frac{\eta_{sp}}{c} = \frac{\eta_{rel} - 1}{c}$

Inherent viscosity : $c \quad \eta_{inh} = \frac{In\ \eta_{rel}}{c}$

Intrinsic viscosity : $[\eta] = \left(\frac{\eta_{sp}}{c}\right)_{c=o} = (\eta_{inh})C = 0$

6. Relative method

Dilute solution viscometry

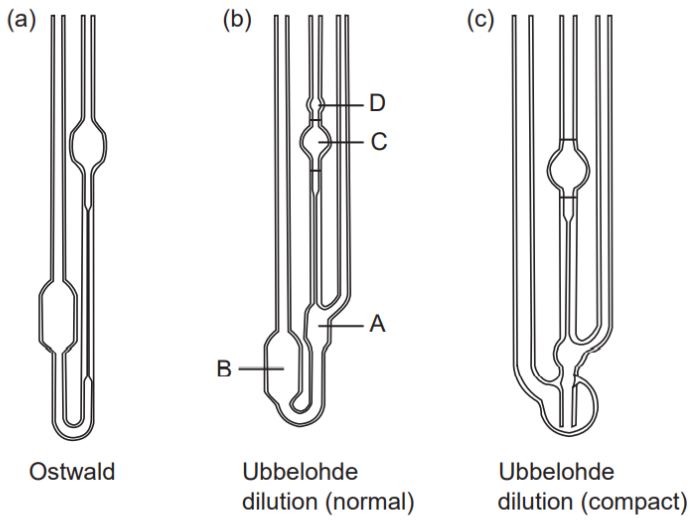


Figure 5 Sketch of the (a) Oswald viscometer, (b) Ubbelohde dilution viscometer in the normal form, and (c) compact form.

$$\left(\frac{M^2}{[\eta]}\right)^{1/3} = A_\eta + B_\eta M^{1/2}$$

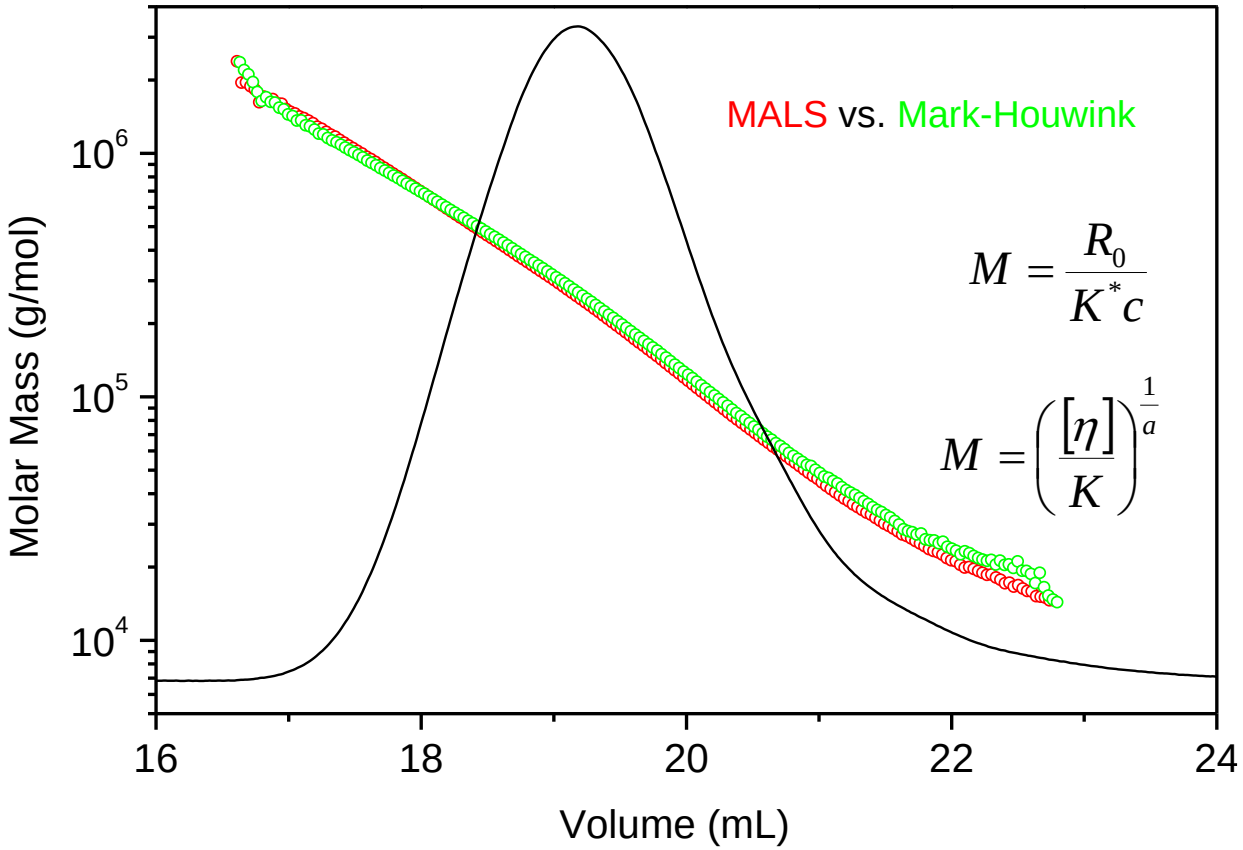
$[\eta] = KM^a$ **Mark–Houwink– Sakurada equation**

- K, a = constants of Mark-Houwink equation for given polymer, solvent and temperature; M = molar mass
- Traditional method of the determination of molar mass
- Viscosity average (M_v) close to weight-average (M_w)

6. Relative method

Dilute solution viscometry

Molar Mass from Mark-Houwink Equation



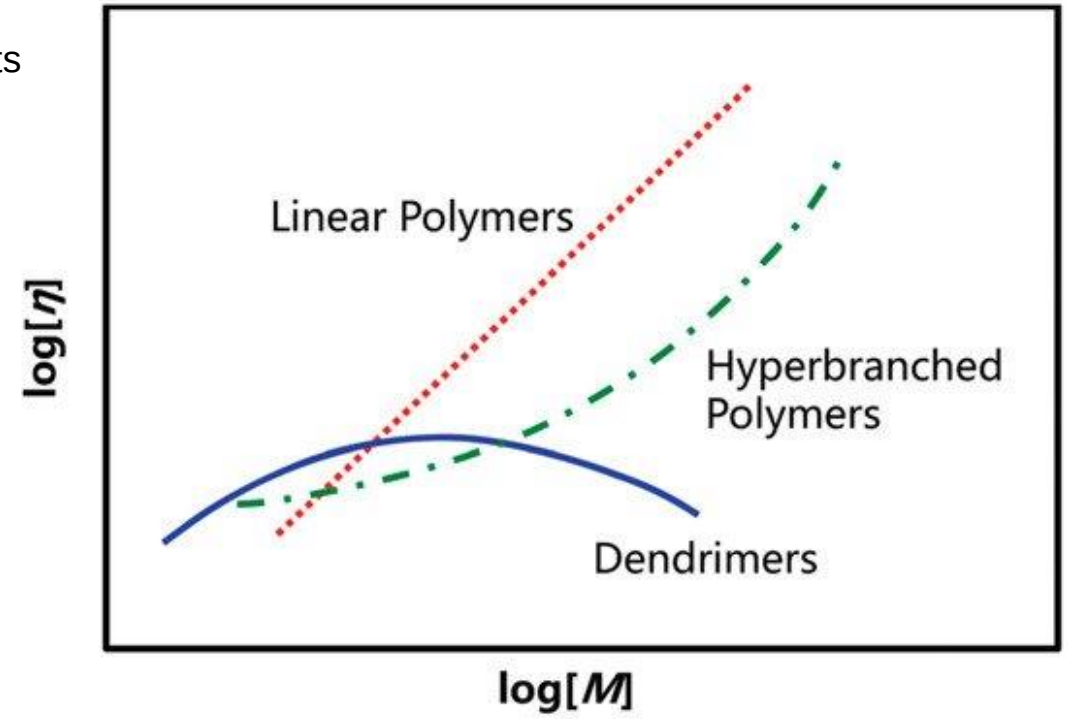
Molar mass versus elution volume plots of linear polystyrene by MALS and calculated from Mark-Houwink equation.

6. Relative method

Dilute solution viscometry

Exponent of Mark-Houwink equation

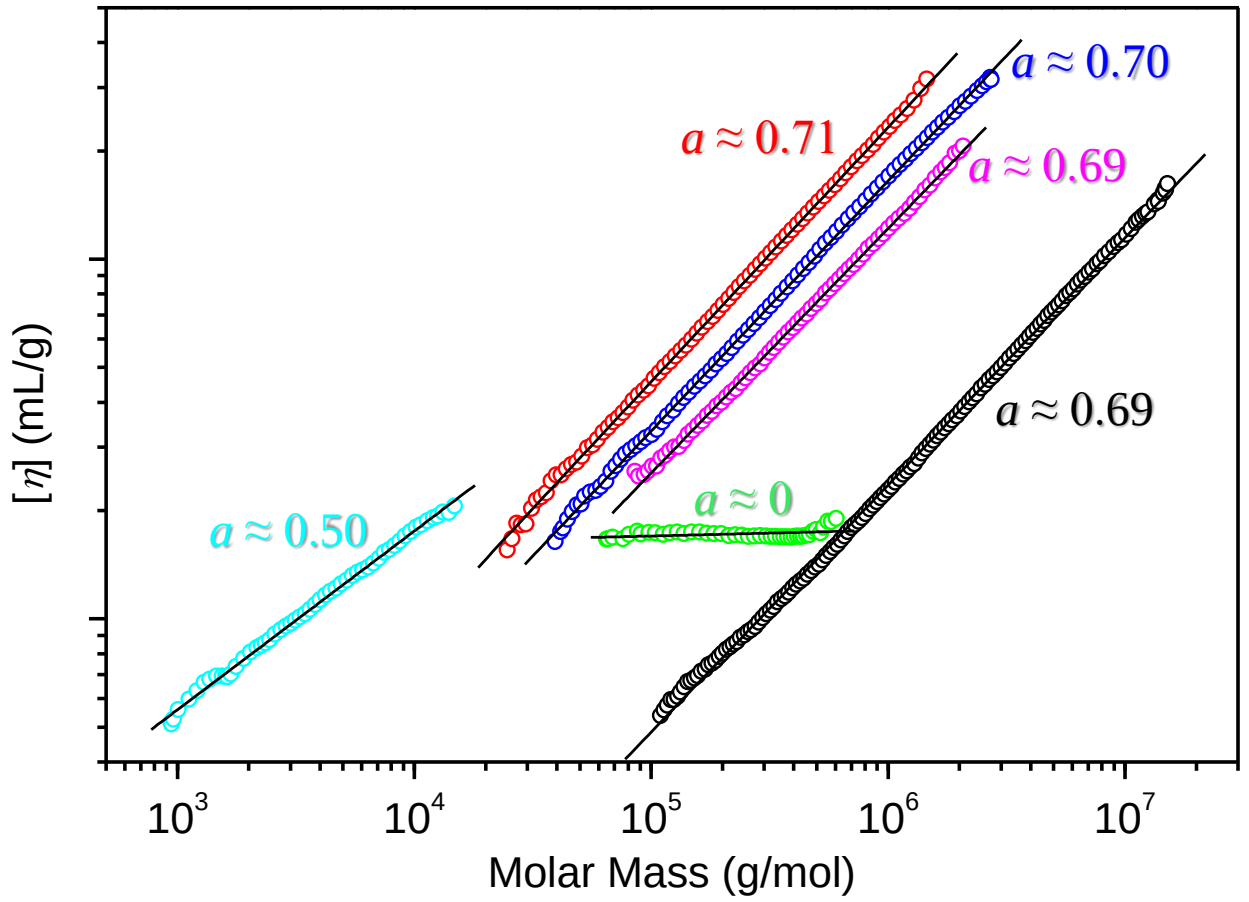
- Linear macromolecules in thermodynamically good solvents: $a \approx 0.7$
- Linear macromolecules in thermodynamically poor solvents: $a \approx 0.5$
- Oligomers: $a \approx 0.5$
- Hard spheres: $a \approx 0$
- Extended chains: $a \approx 0.8$ to ≈ 1.5
- Linear polymers have linear MH plots
- Curved plots indicates branching



Molecules **2018**, *23*(3), 657

6. Relative method

Dilute solution viscometry

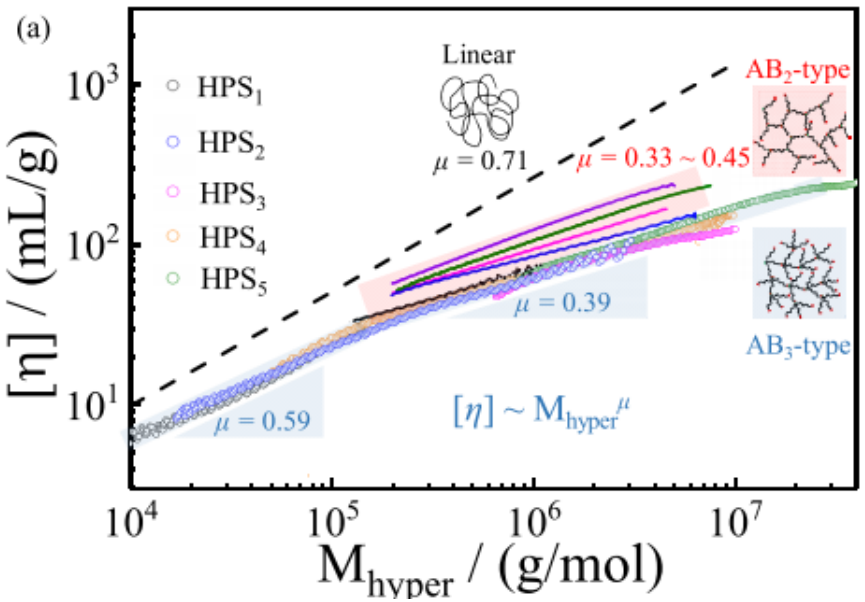


Mark-Houwink plots of epoxy resin, linear polystyrene, linear poly(methyl methacrylate, linear poly(benzyl methacrylate), linear poly(iBuPOSSMA) and star-branched poly(isobutyl methacrylate).

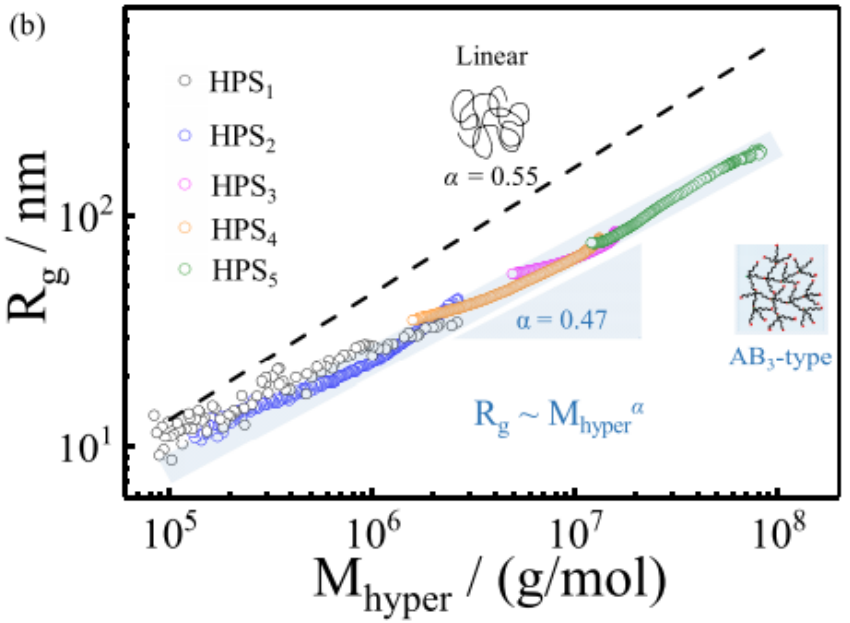
6. Relative method

Dilute solution viscometry

How Does the Branching Effect of Macromonomer Influence the Polymerization, Structural Features, and Solution Properties of Long-Subchain Hyperbranched Polymers?



Scaling of intrisic viscosity

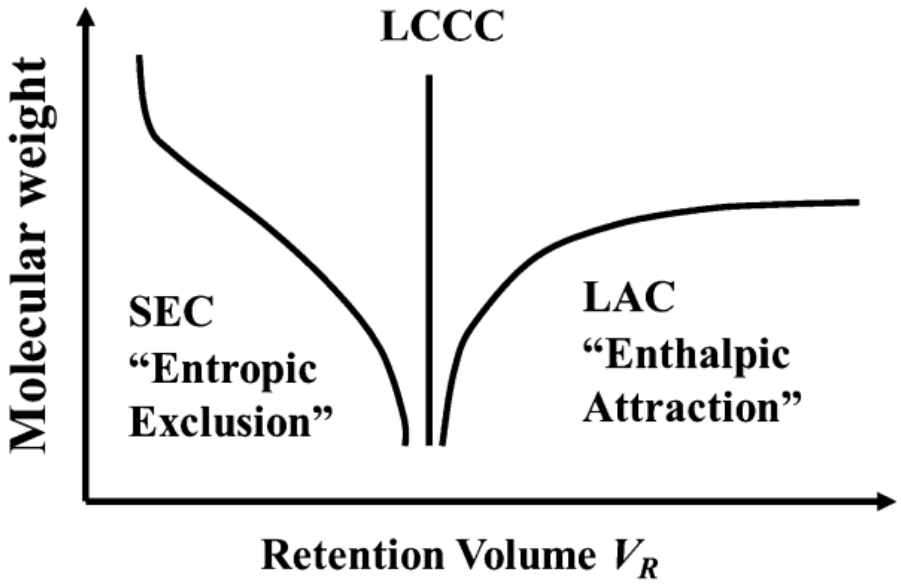


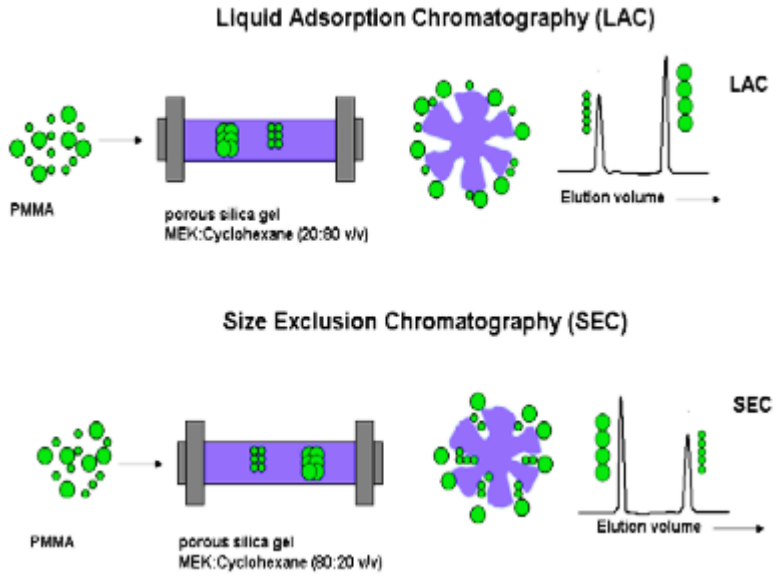
Scaling of the Radius of Gyration

https://doi.org/10.1021/acs.macromol.8b02364

6. Relative method

Types of liquid chromatographic separations





Size Exclusion Chromatography (SEC)

Liquid Chromatography under Critical Conditions (LCCC)

Liquid Adsorption Chromatography (LAC)

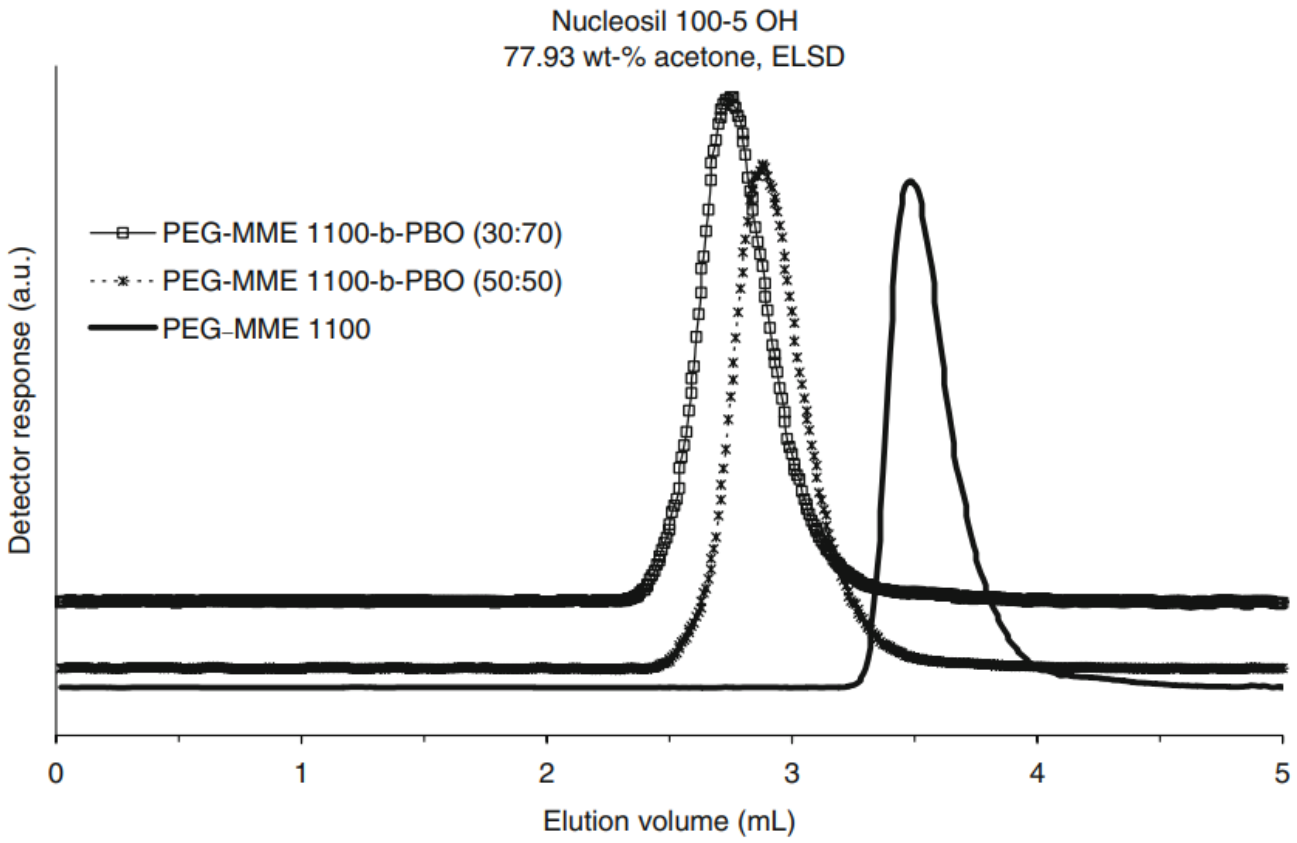
Soft Matter, 2016, 12, 5245

5. Relative method

Types of liquid chromatographic separations

Liquid Chromatography under Critical Conditions (LCCC)

Fig. 3 LCCC (at the CAP for EO) of PEG-MME and the corresponding diblock copolymers with butene oxide (BO) (exclusion conditions for the BO block). *PBO* poly(butene oxide)

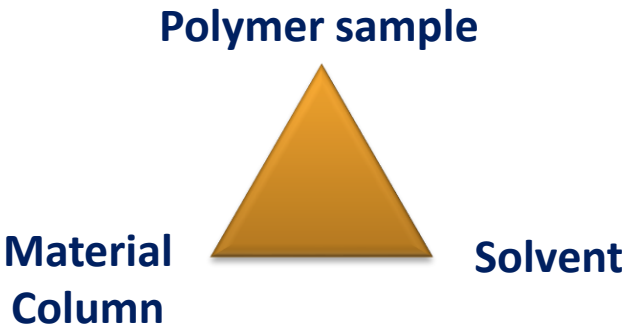


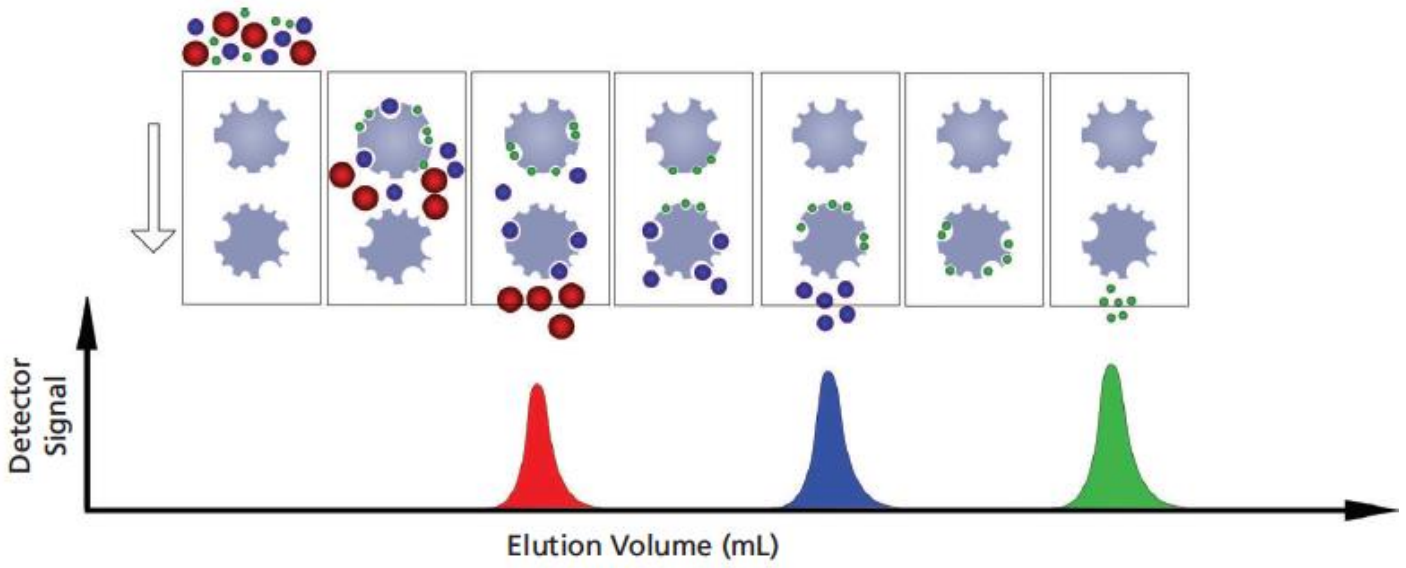
Anal Bioanal Chem (2009) 393:1797–1804

6. Relative method

Size Exclusion Chromatography (SEC)

molar mass determination based on separation according to size



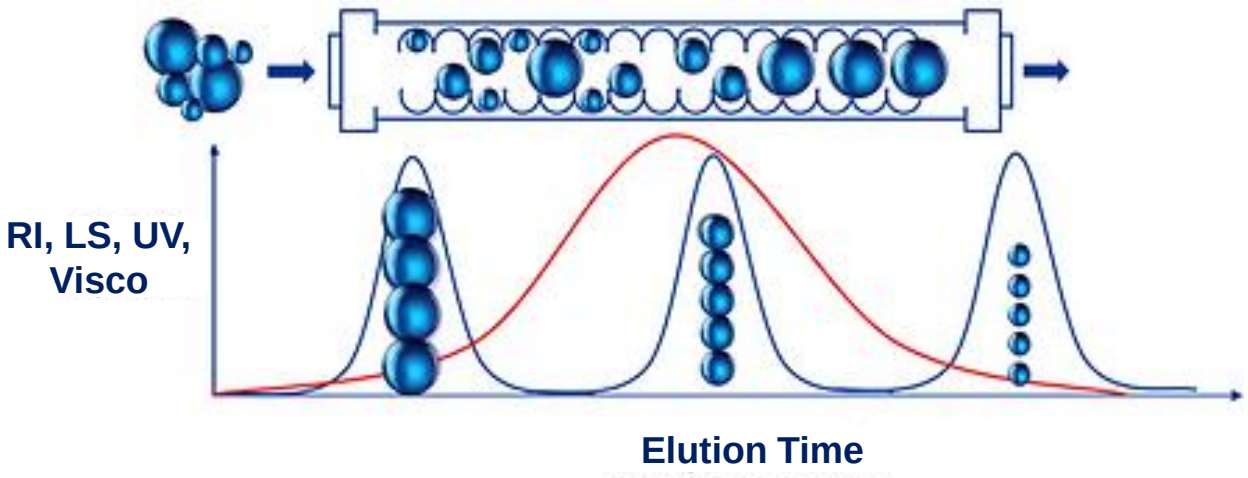


- **Large particles** cannot enther gel and **are excluded**. They have less volume to traverse and **elute sooner**

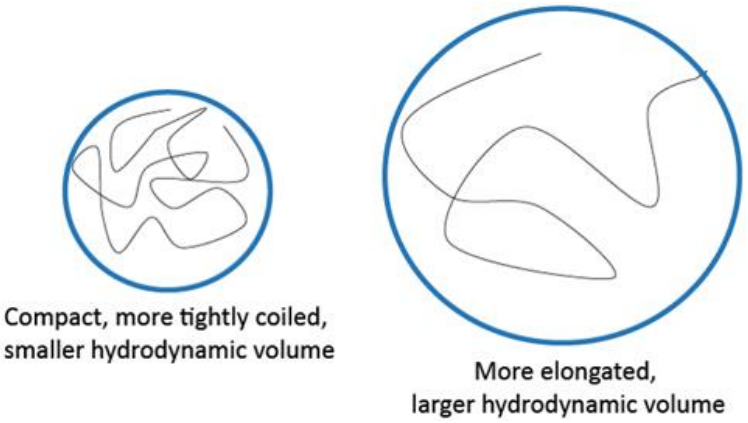
- **Small particles** can enter gel and have more volume to traverse. They **elute later**

6. Relative method

Size Exclusion Chromatography (SEC)

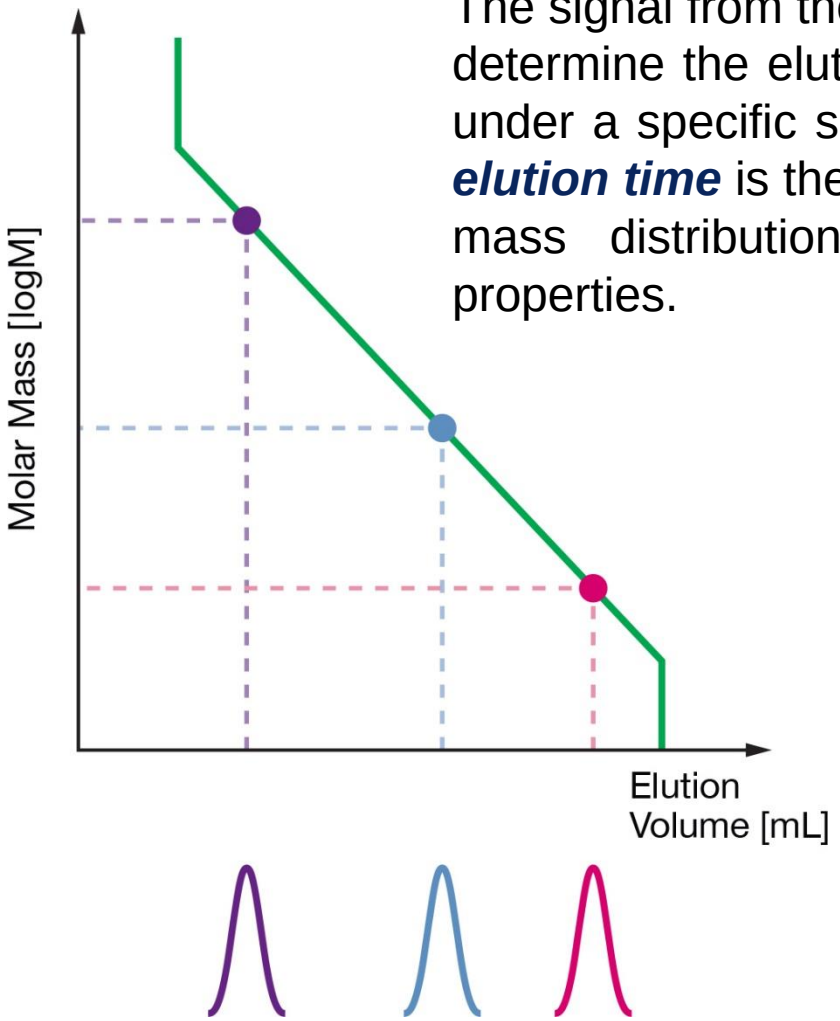


SEC is separating polymers by their **hydrodynamic volume** or **hydrodynamic radius** – which is affected by various things, in particular the polymer (chemistry and structure), solvent, solvent/polymer interactions, and temperature.



6. Relative method

Size Exclusion Chromatography (SEC)- Calibration curve



The signal from the ***concentration detector*** in SEC is used to determine the elution time of ***known molar mass standards*** under a specific set of conditions. A plot of ***molar mass vs. elution time*** is then generated and used to estimate the molar mass distribution of actual samples from their elution properties.

The column calibration method assumes that the sample of interest

- has the same conformation as calibration standards
- has the same density as standards
- does not interact with the column packing

6. Relative method

What is SEC-MALS?

- **SEC** = **S**ize-**E**xclusion **C**hromatography

  Size-Exclusion Chromatography (SEC) is a chromatographic method in which molecules are separated based on their size, or, in more technical terms, their hydrodynamic volume.

- **MALS** = **M**ulti-**A**ngle **L**ight **S**cattering

Two modes of operation

$$I_s(\theta) \propto c \times M \times \left(\frac{dn}{dc}\right)^2$$

- A batch experiment measures an unfractionated sample.

  → Only the weight-averaged molar mass M_w, z-averaged RMS radius R_z, and potentially A_2 will be determined.

  → No information about the polydispersity of the sample can be obtained.

- SEC allows the sample to be chromatographically separated.

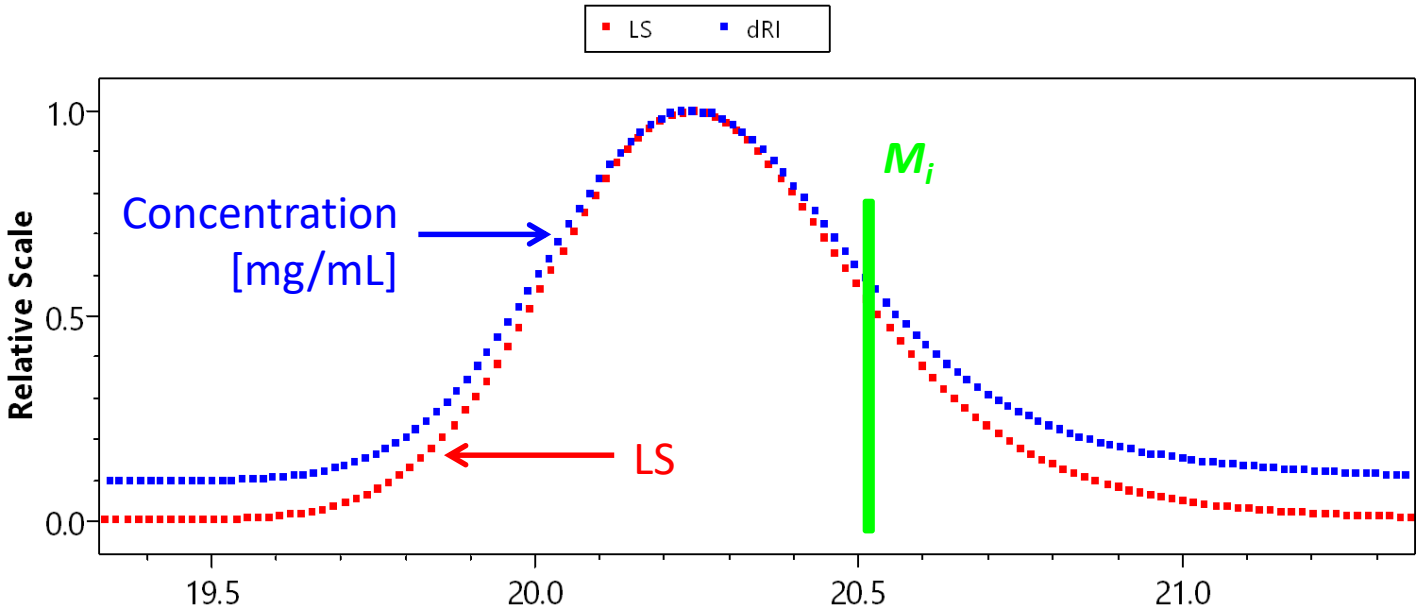
  → Molar Mass and RMS Radius moments and distributions can be assessed.

6. Relative method

SEC-MALS-UV/RI method

- SEC provides separation and the molar mass is measured by online MALS and concentration detectors. Molar mass moments (M_n, M_w, M_z), radius moments, dispersity, conformation

$$I_s(\theta) \propto c \times M \times \left(\frac{dn}{dc}\right)^2$$



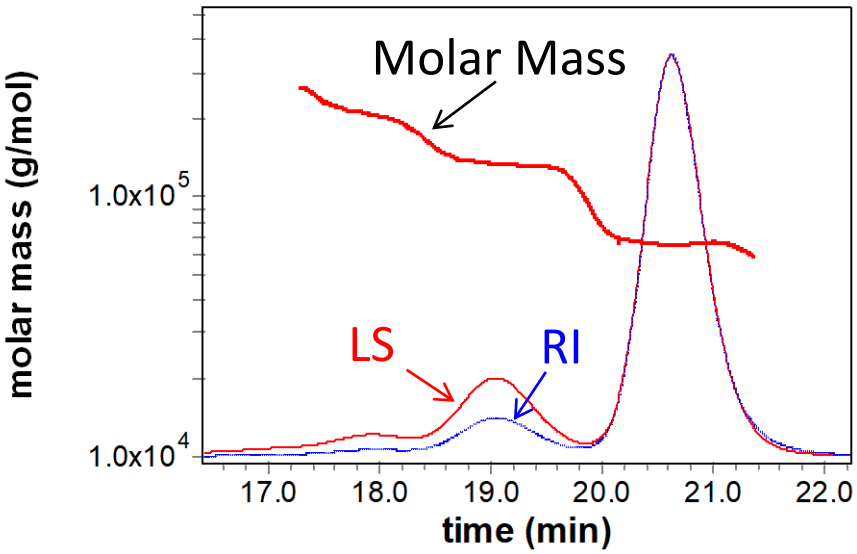
6. Relative method

SEC-MALS-UV/RI method

Molar Mass at each elution slice is measured

Proteins

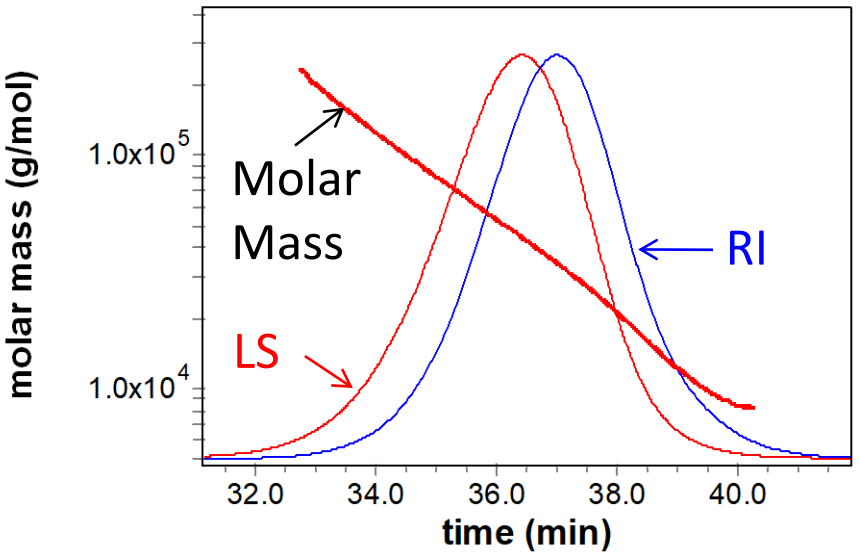
(discrete populations)



BSA

Polymers

(continuous distribution)

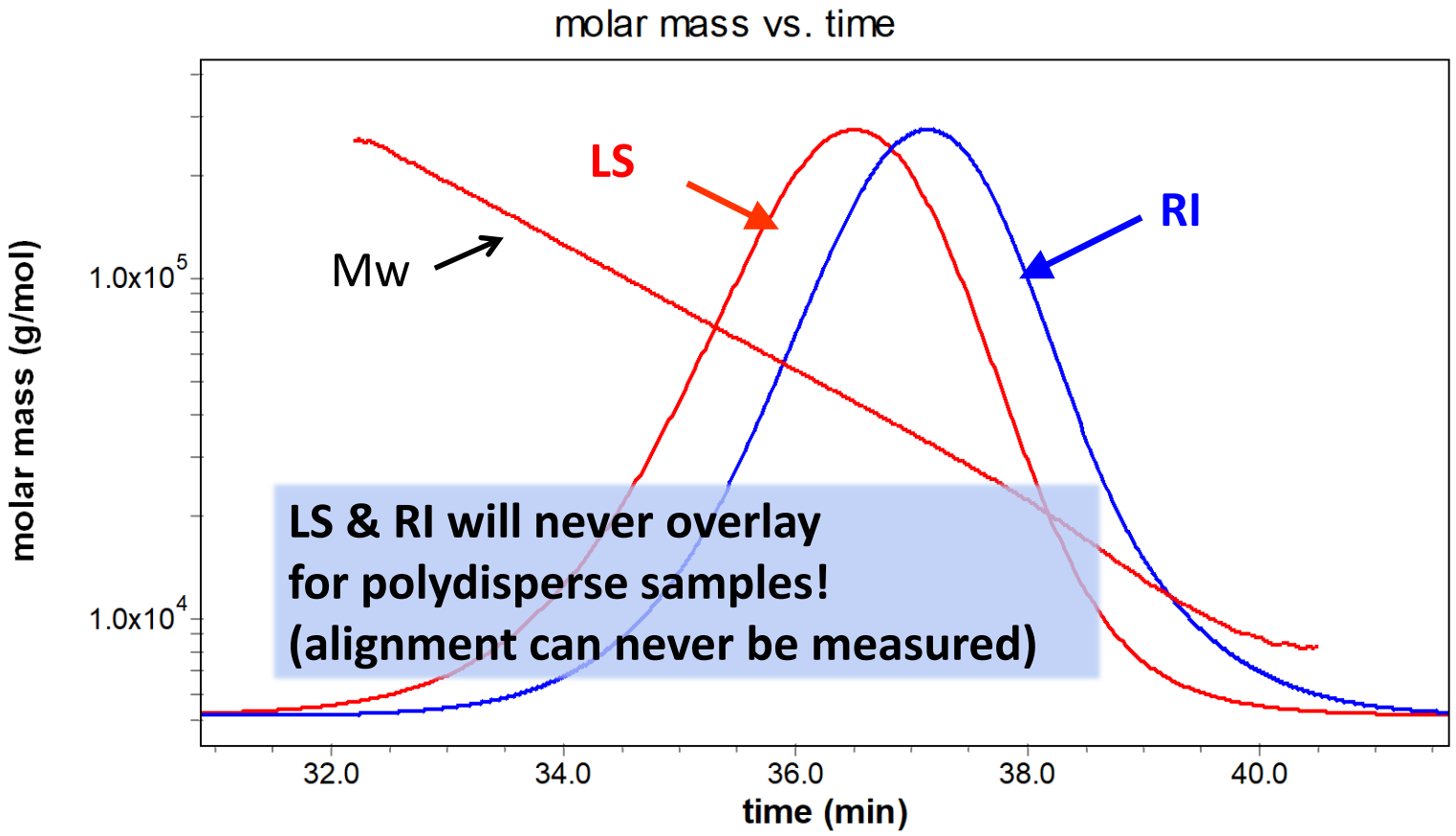


Dextran

30

5. Relative method

SEC-MALS-UV/RI method



6. Relative method

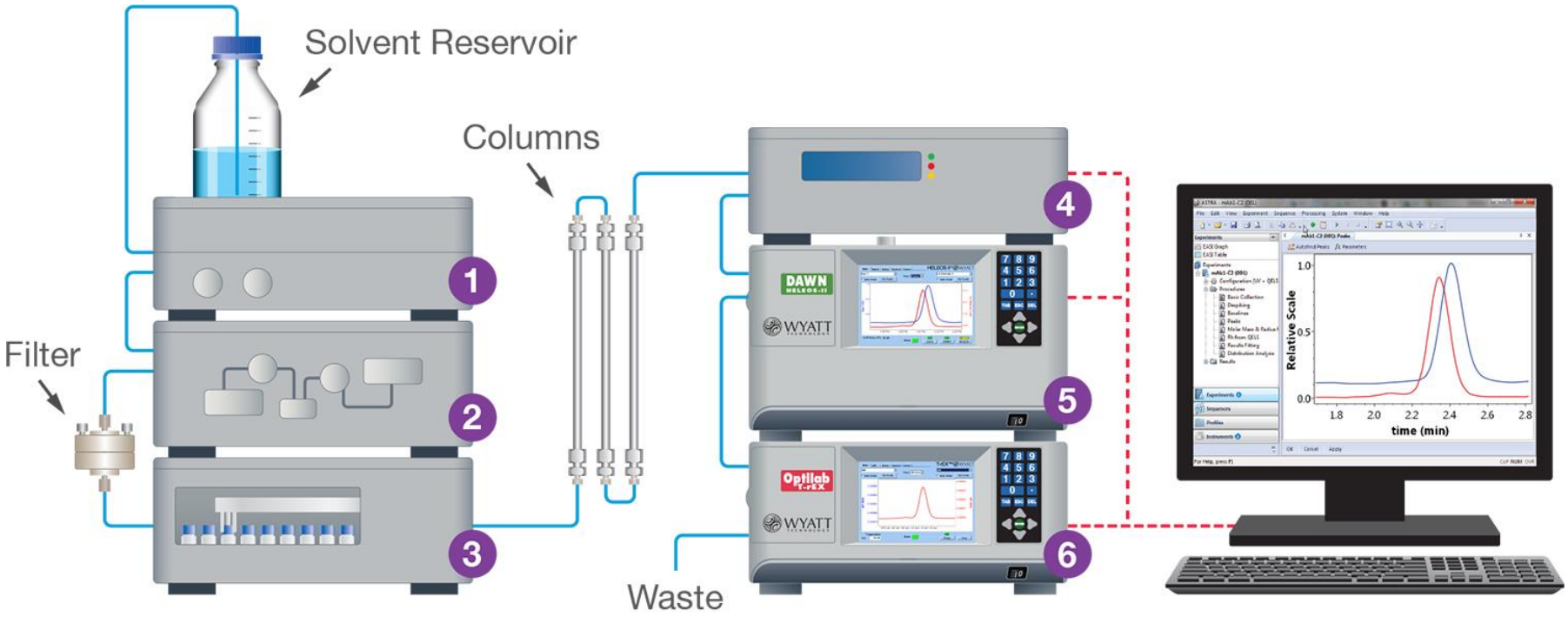
SEC-MALS-UV/RI method

- Knowledge of *dn/dc* is necessary for
  - → Molar mass determination from light scattering measurements
  - → Determining concentrations by an RI detector
  - → Analog RI calibration
- The value of *dn/dc* depends on
  - → Polymer composition
  - → Solvent
  - → Molar mass
  - → Laser wavelength
- The *dn/dc* can be obtained from
  - → The literature
  - → Direct measurement with an RI detector (online or offline)
- For greatest accuracy, the dn/dc used in light scattering experiments should have been obtained for your polymer, in your solvent, and at the wavelength of your LS detector.
- dn/dc describes the change in the refractive index of a solution as a function of solute concentration.
- dn/dc has units of mL/g; it expresses how much the refractive index of a solution theoretically increases for every g of solute contained in a 1 ml final volume of solution.
- The dn/dc of a polymer solution should be measured at polymer concentrations appropriate for chromatography conditions; some non-linearity can occur at very high solute concentrations.
- To determine molar mass by LS, dn/dc needs to be known
  - → For your solute
  - → In your solvent
  - → At the wavelength of your LS detector
  - → Even when you are not using a concentration detector!

32

6. Relative method

Typical SEC-MALS-hardware setup

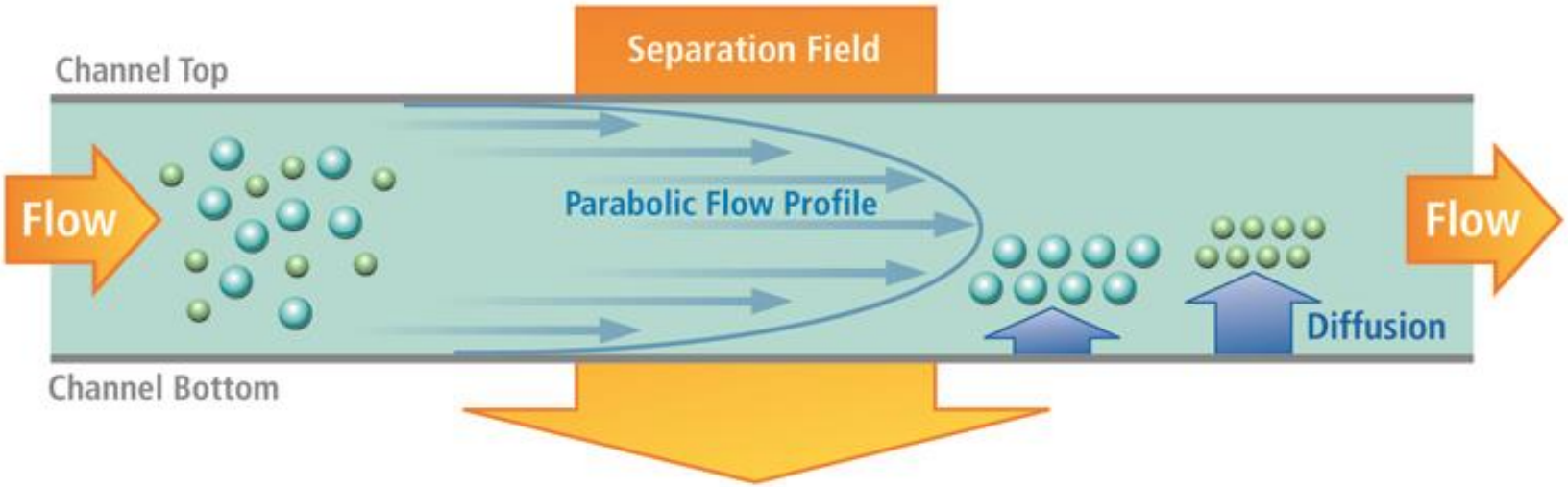


1. Degasser
2. Pump
3. Autosampler or Manual Injector
4. UV Detector
5. Multi-Angle Light Scattering Detector
6. Differential Refractive Index Detector

ASTRA Software

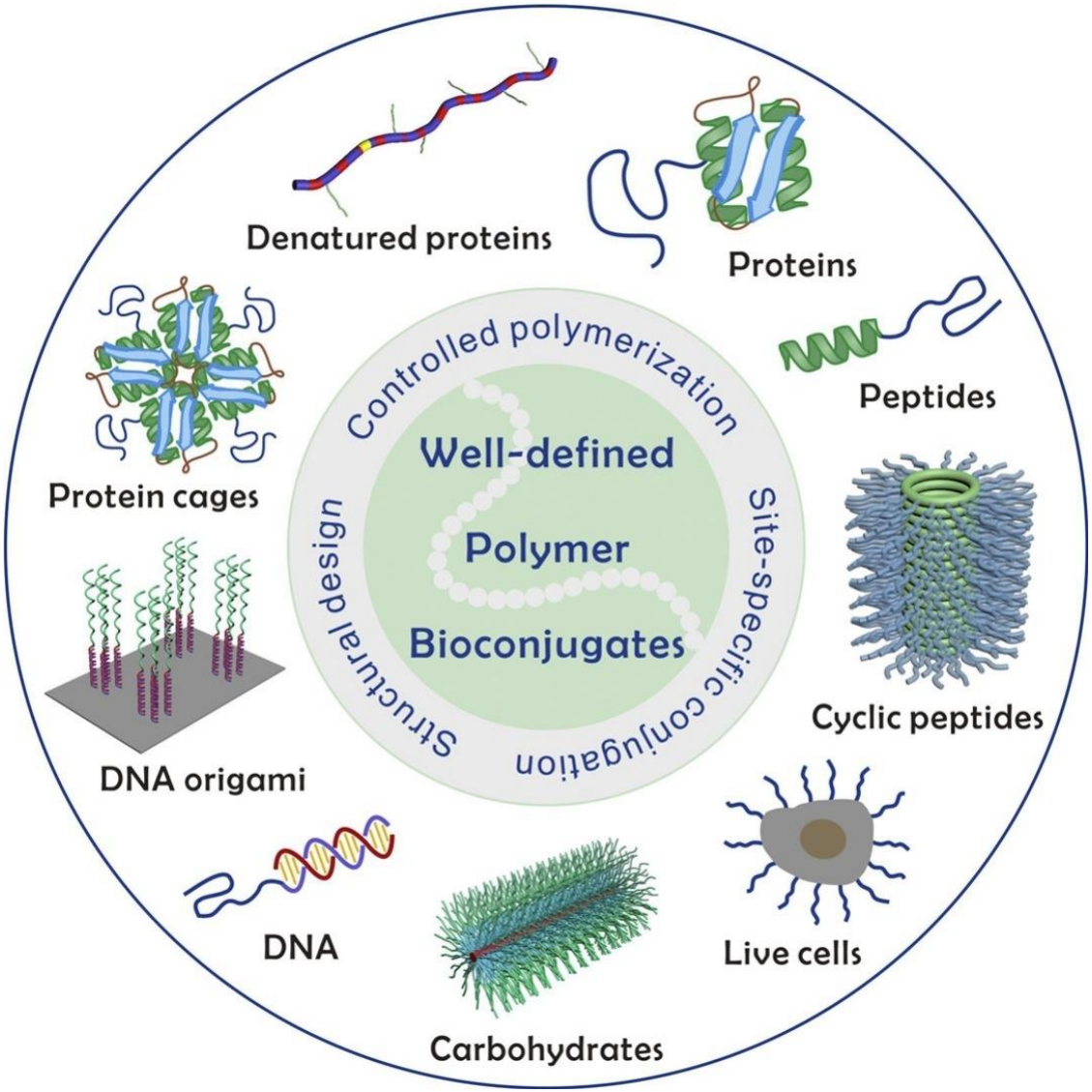
6. Relative method

Field-flow fractionation (FFF)



It is a separation technique in which a field (thermal, electric, magnetic, hydraulic, gravitational, ...) is applied to a diluted suspension in a fluid or to a solution pumped through a long and narrow channel, perpendicular to the direction of the field, in order to cause the separation of particles present in the fluid, depending on their differing "mobilities" under the force exerted by the field. The FFF method is unique to other separation techniques because it can separate materials over a wide colloidal size range while maintaining high resolution. Although FFF is an extremely versatile technique, there is no "one size fits all" method for all applications.

7. Polymer bioconjugates: Modern design concepts toward precision hybrid materials



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7. Polymer bioconjugates: Modern design concepts toward precision hybrid materials

1970

1975 The model of synthetic polymer-drug conjugates proposed by Ringsdorf [7]

1977 First example of PEG conjugation to a protein[8]

1987 PNIPAM-antibody conjugates for bioseparation[9]

1990

1990 First protein-polymer drug approved by FDA[140]

1995 Site-specific conjugation of streptavidin with PNIPAM to control protein activity[12]

1995 ATRP discovered by Matyjaszewski & Sawamoto[24,25]

1998 RAFT polymerization developed[26]

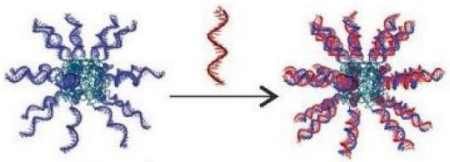
1997 PEG conjugated to the surface of red blood cells[461]

2000

2002 In situ ATRP from cellulose fibers[438]

2003 First example of sequence defined synthesis aided by DNA complementarity[399]

2007 DNA block copolymer micellar assembly[422]



2002 Self-assembly of lipase-polystyrene conjugates[300]

2002 Photoresponsive polymer-enzyme switches[158]

2005 In situ ATRP growth of polymers from proteins[77]

2007 In situ RAFT polymerization from proteins[133]

2008

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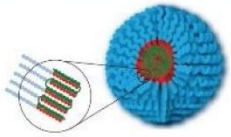
7. Polymer bioconjugates: Modern design concepts toward precision hybrid materials

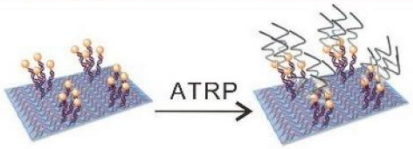
2012 Cooperative synthesis of polymeric nanoparticles programmed by DNA[402]



2013 Cyclodextrin-polymer conjugates for preparing nearly monodisperse colloidal nanocrystals[448]

2013 Heparin-like polymers for protein conjugation[156]
2013 Janus cyclic peptide-polymer nanotubes[297]
2013 Interfacial assembly of BSA-PNIPAM conjugates[319]

2016 Synergistic assembly of sequence defined polymer-DNA cages[413]
2016 In situ ATRP from DNA origami[418]



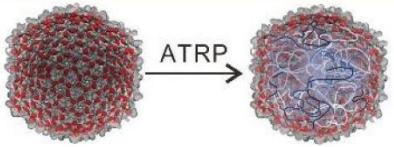
2018 DNA-imprinted polymer nanoparticles[406]
2018 Superlattices with DNA-nanoparticle assembly[414]

2013

2016

2018

2010 Denatured proteins conjugated with PEG for surface coating of quantum dots[219]
2012 Zwitterionic polymers for protein conjugation[152]
2012 Site-specific ATRP from the interior cavity of protein cages[253]

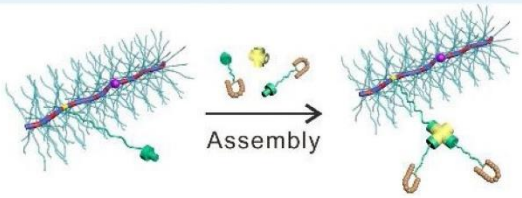


2014 Solid-phase ATRP for DNA-polymer biohybrids[115]
2015 Shaping a single strand polymer on DNA origami[415]

2016 Site-specific topological protein-polymer hybrids[205]

2016 Cellulose-polymer conjugates for 1D nanocrystals[449]
2016 In situ ATRP from living cell surfaces[467]
2017 In situ RAFT polymerization from cell surfaces[469]

2018 Self-assembly of cyclic peptide-polymer conjugates into tubisomes[298]
2018 Muscle-inspired anisotropic actuators base on peptide-polymer conjugates[393]
2019 Reactive oxygen species-triggered morphology transformation of peptide-polymer conjugates[278]
2020 Precision brush polymers from unfolded proteins[233]



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