Leibniz-Institut für Polymerforschung Dresden

Polymers in Solution

Dresden, 30th November 2022

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Bioactive and Responsive Polymers
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Leibniz-Institut für Polymerforschung Dresden e.V.

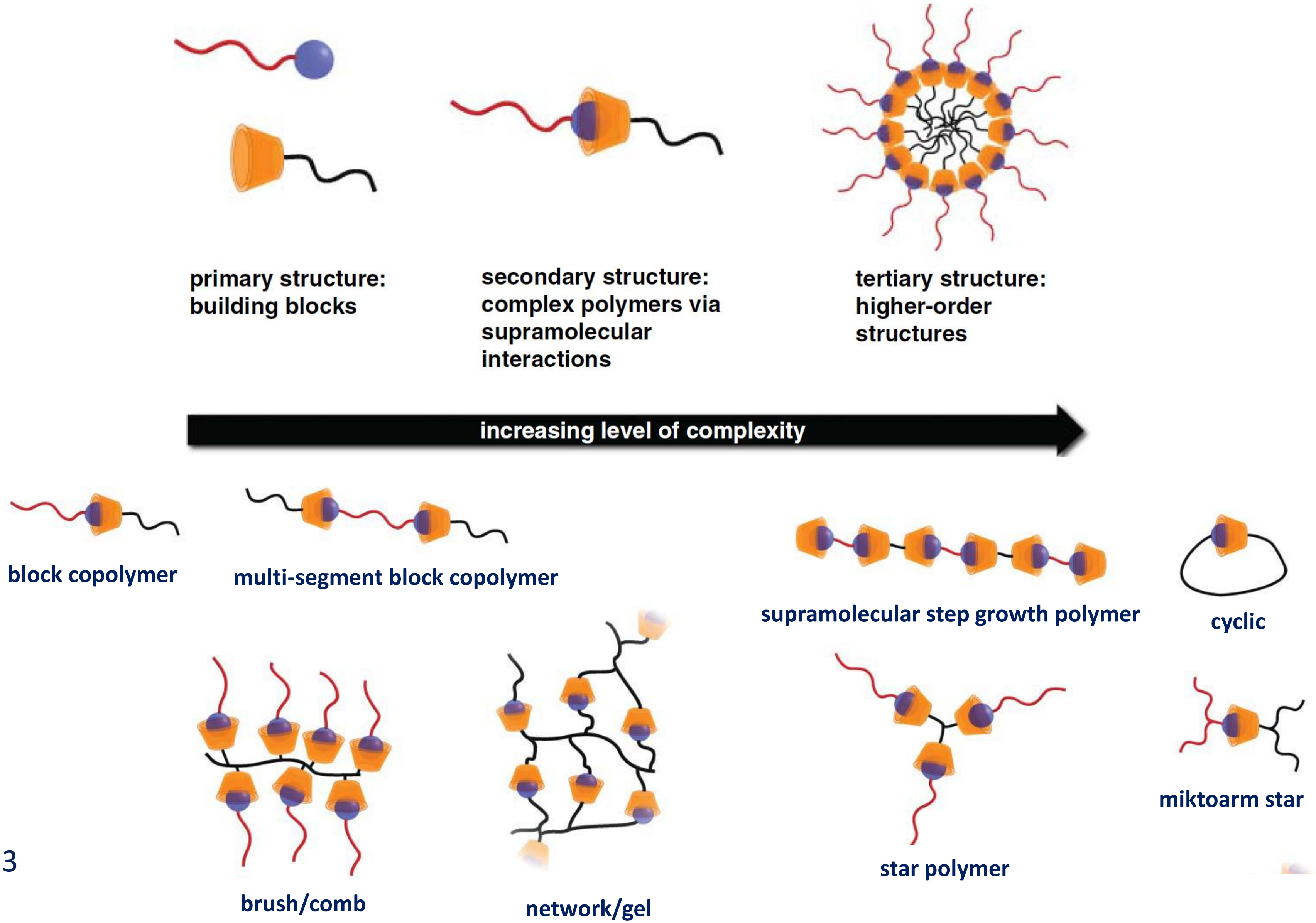
DRESDEN concept

Leibniz Gemeinschaft

Macromolecular Self-Assembly and interaction with biomacromolecules: Characterization

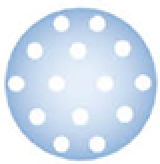
1. Supramolecular Approach to Macromolecular Self-Assembly

SUPRAMOLECULAR CD SELF-ASSEMBLIES



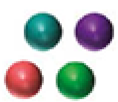
1. Supramolecular Approach to Macromolecular Self-Assembly: Kind of nanoparticles based on material

INORGANIC

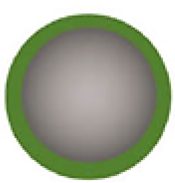


Silica nanoparticles

Gold nanoparticles

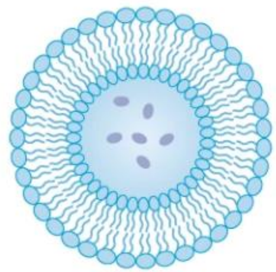


Quantum dots

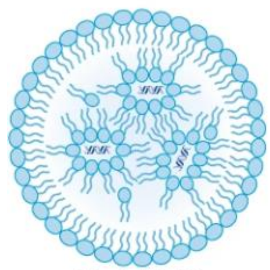


Magnetic nanoparticles

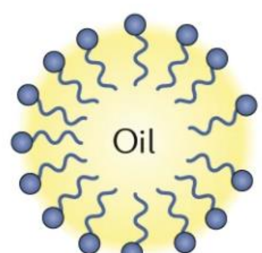
LIPIDS



Liposome

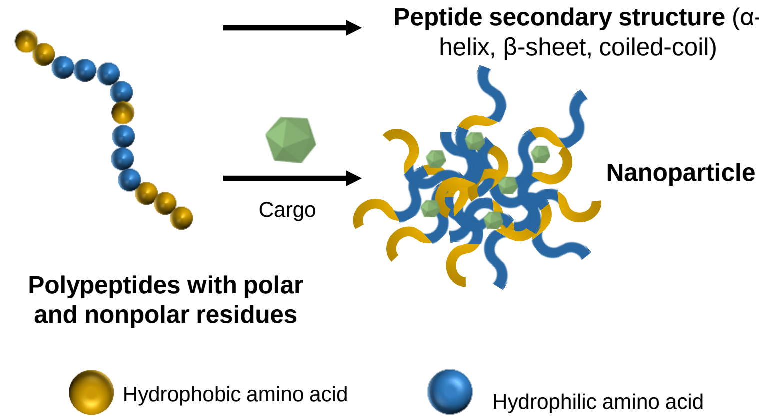


Lipid NP

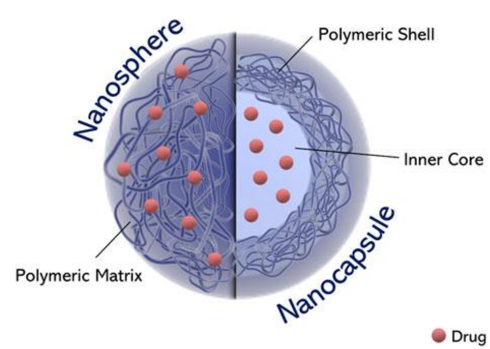


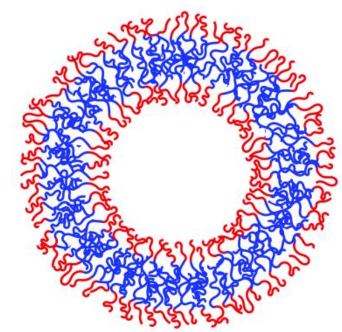
Emulsion

PEPTIDES

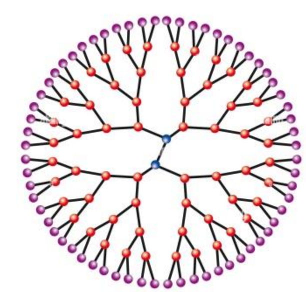


POLYMERS



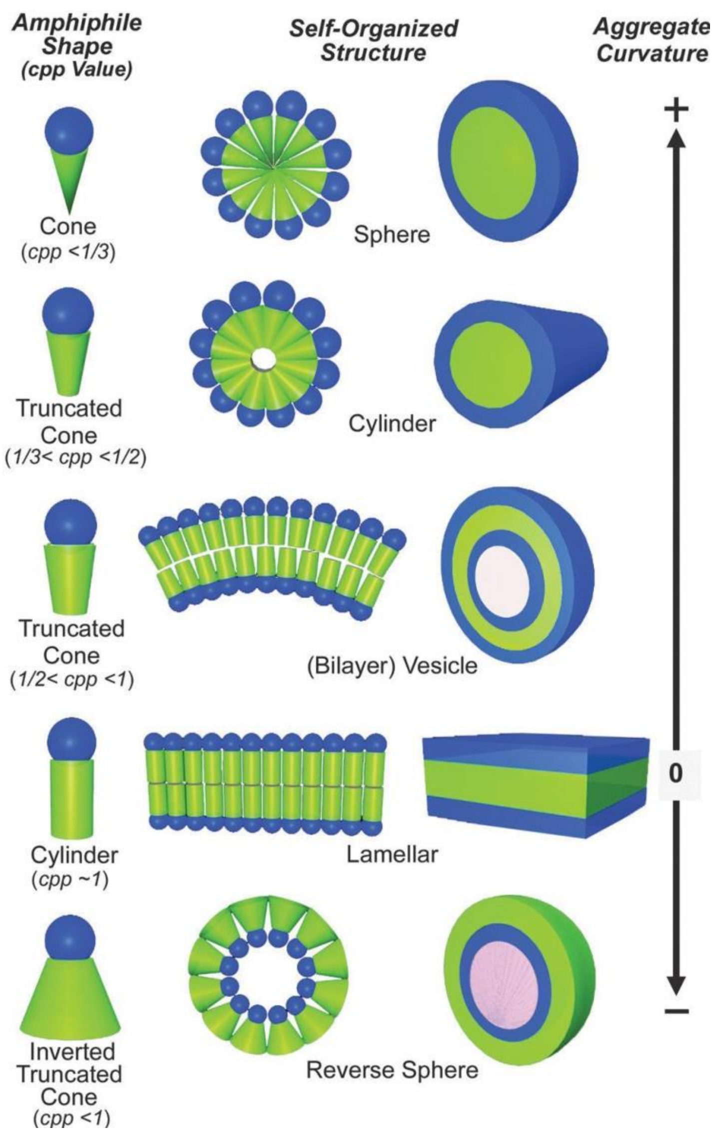


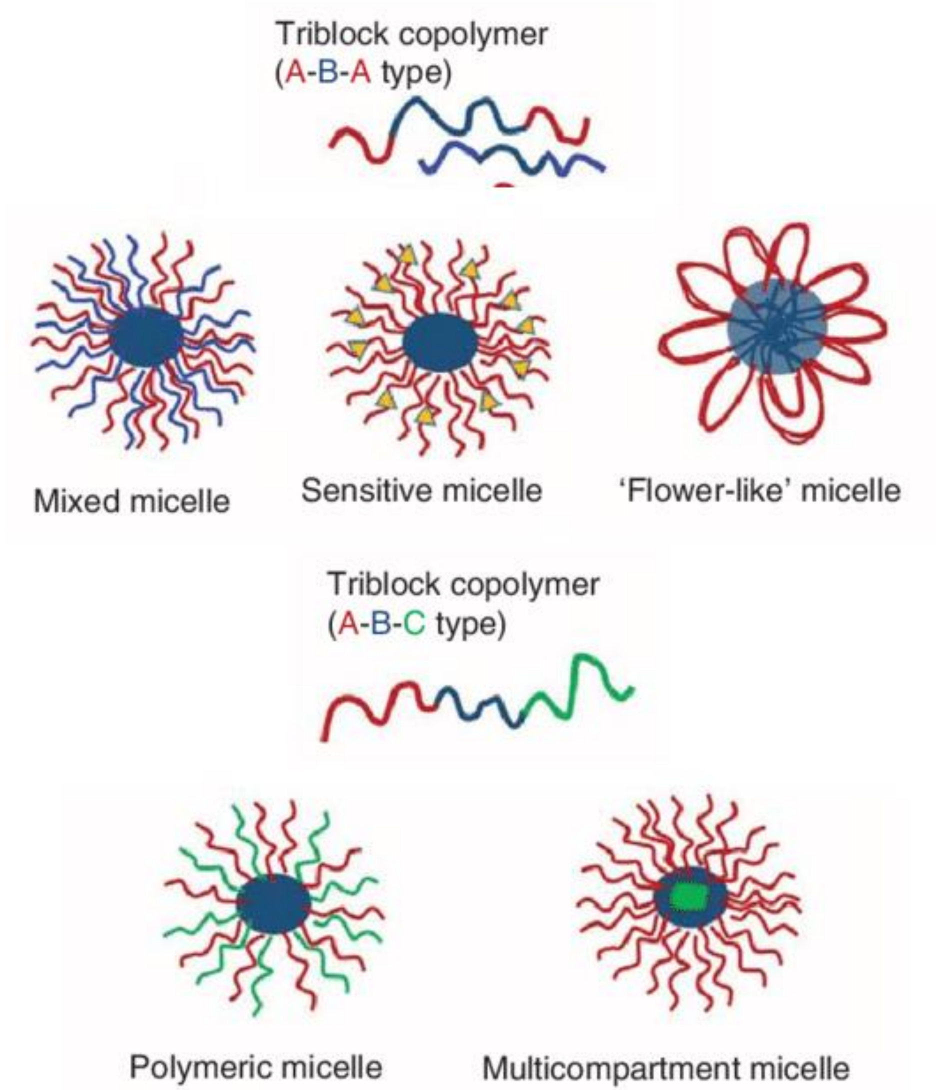
Polymersome



1. Supramolecular Approach to Macromolecular Self-Assembly

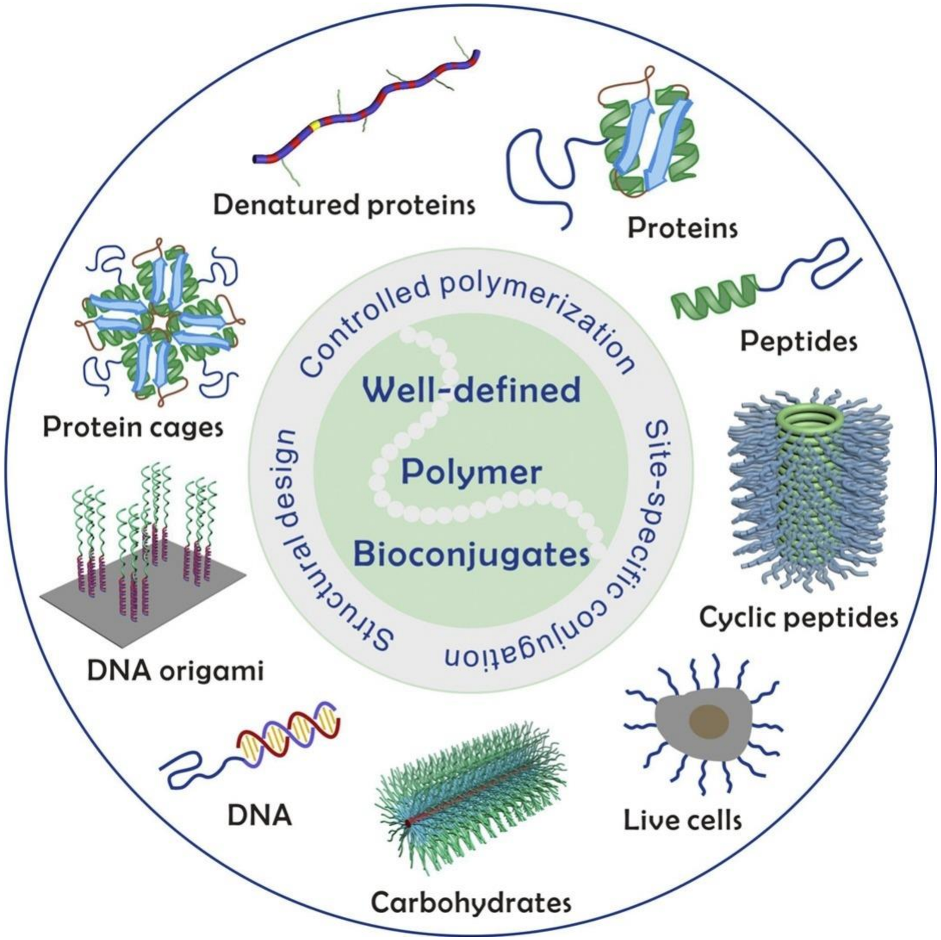
HIGHER ORDER ASSEMBLIES ARCHITECTURES TOWARD NANOSTRUCTURES





1. Supramolecular Approach to Macromolecular Self-Assembly

BIOCONJUGATES- Bio and mutifunctional polymer architectures

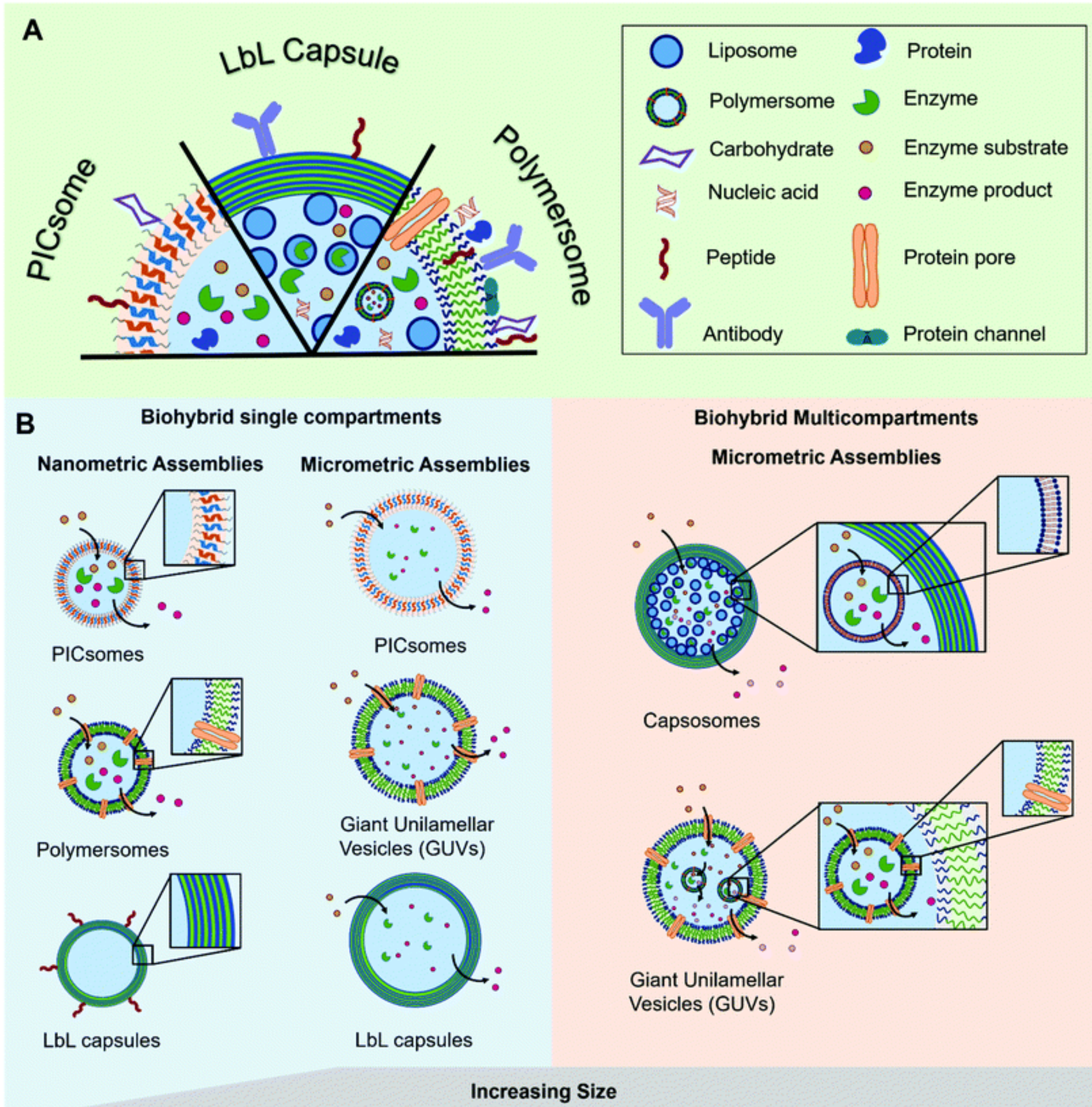


(1) Reproducibility of bioconjugate production,
(2) Activity of biomolecules attached to bioconjugate,
(3) Long-term stability of the bioconjugate
(4) Level of control over chemical and biomodification of the surface
(5) Purity and potential for contamination of bioconjugate

Adequate purification and characterization play a fundamental role

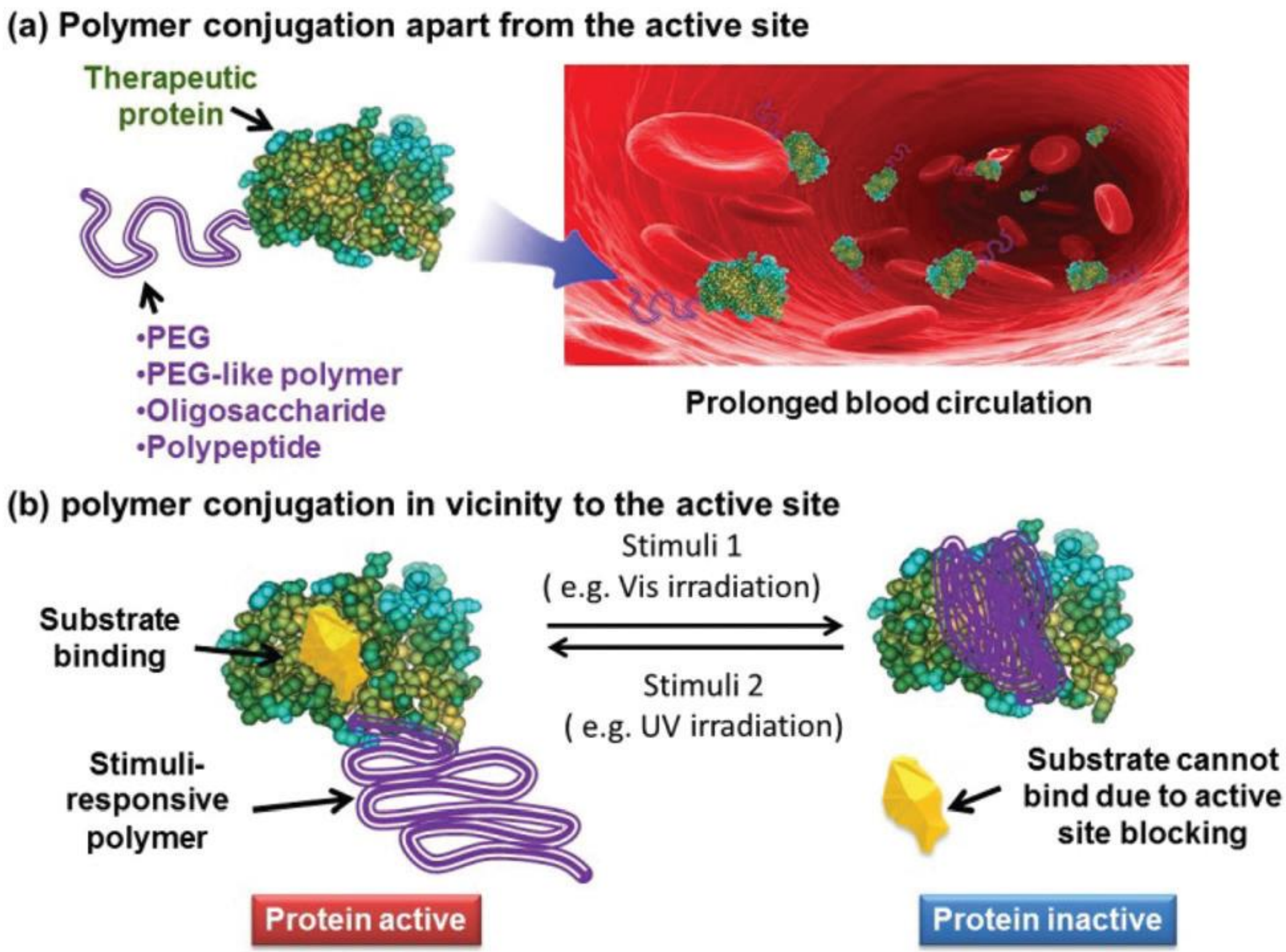
https://doi.org/10.1016/j.progpolymsci.2020.101241

1. Supramolecular Approach to Macromolecular Self-Assembly



DOI: 10.1039/D0CP00693A

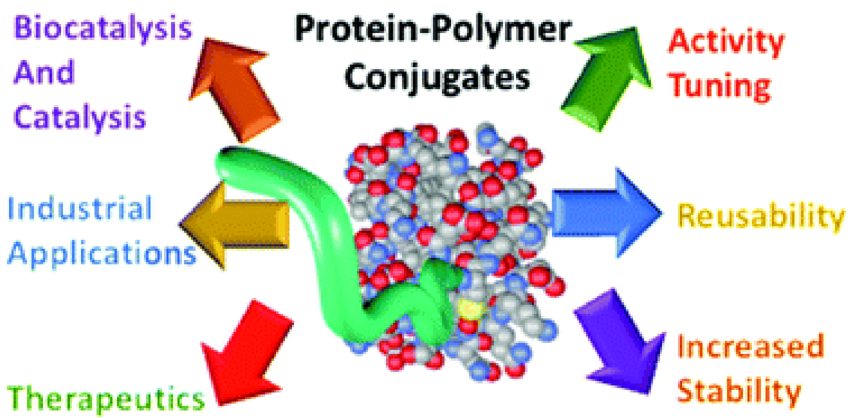
1. Supramolecular Approach to Macromolecular Self-Assembly



Biomater. Sci., 2015,**3**, 214-230

2. Supramolecular Approach to Macromolecular Self-Assembly

BIOCONJUGATES- Bio and mutifunctional polymer architectures



MEASURE

Chemical: (a) activity; (b) stability

Physical: conjugate size Rh, Rg; conformation, interaction or cargo adsortion

Therapeutic properties

Biocomcompatibility, cellular uptake, targeting properties, function

TECHNIQUES

Single polymer bioconjugate ➔

Conformation: pH, temperature, ionic, strenght, solvent

DLS, SEC-MALLS, NMR, viscosity, FTIR, RAMAN, MALDI-TOF, electrophoresis,TGA, DSC, protein assay, fluorescence spectroscopy (Tryptopahn FL), UV-VIS, modeling

Self- assembled nanoparticle

DLS, TEM, SEM, Cryo-TEM, Cryo-SEM, CLSM, FFF, zeta potential, electrophoresis, SAXS, SANS, AFM, modeling, fluorescence spectroscopy, UV-VIS (OD)

2. Supramolecular Approach to Macromolecular Self-Assembly

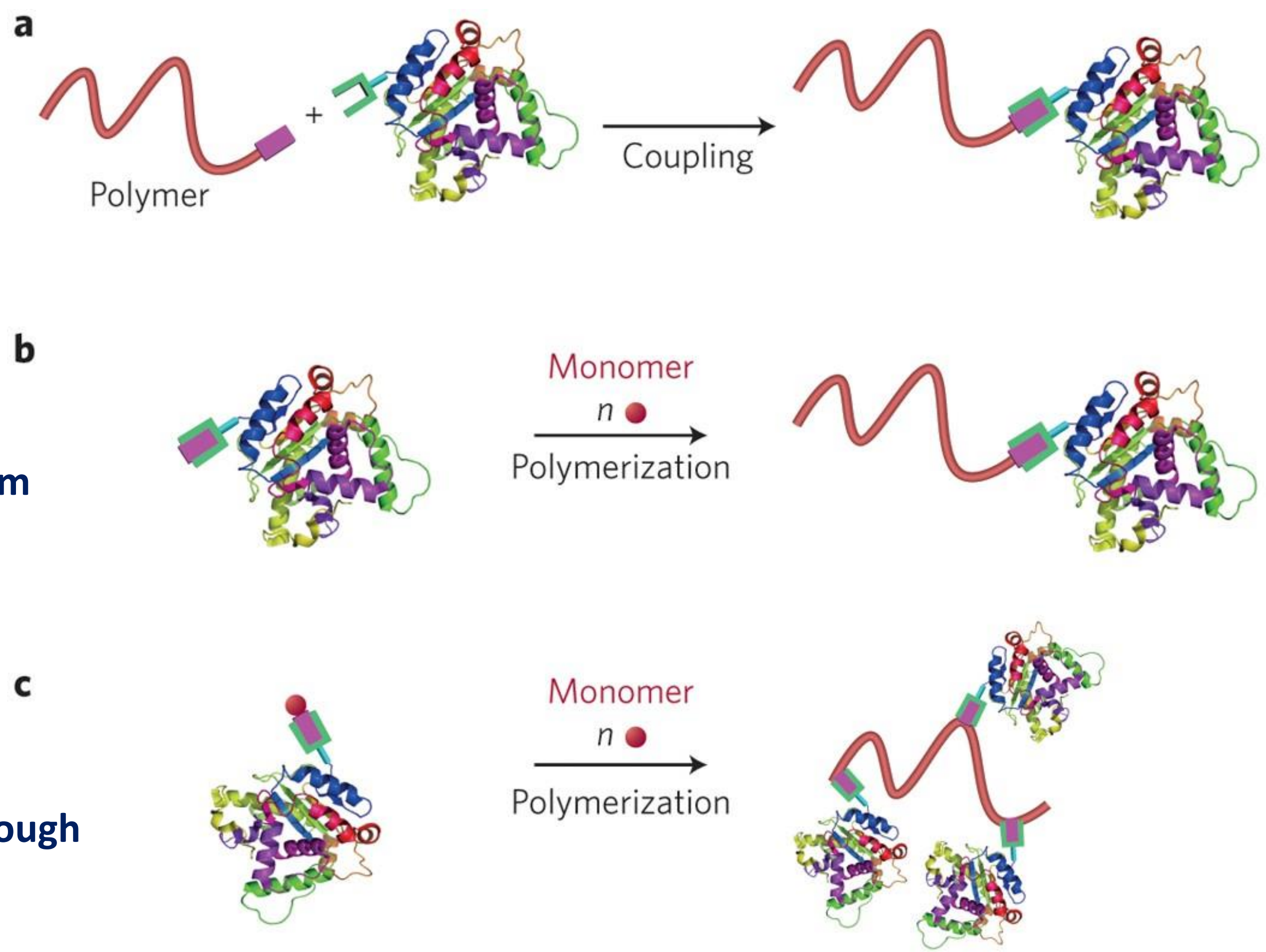
BIOCONJUGATES- Bio and mutifunctional polymer architectures

SYNTHETIC METHODS

Grafting to

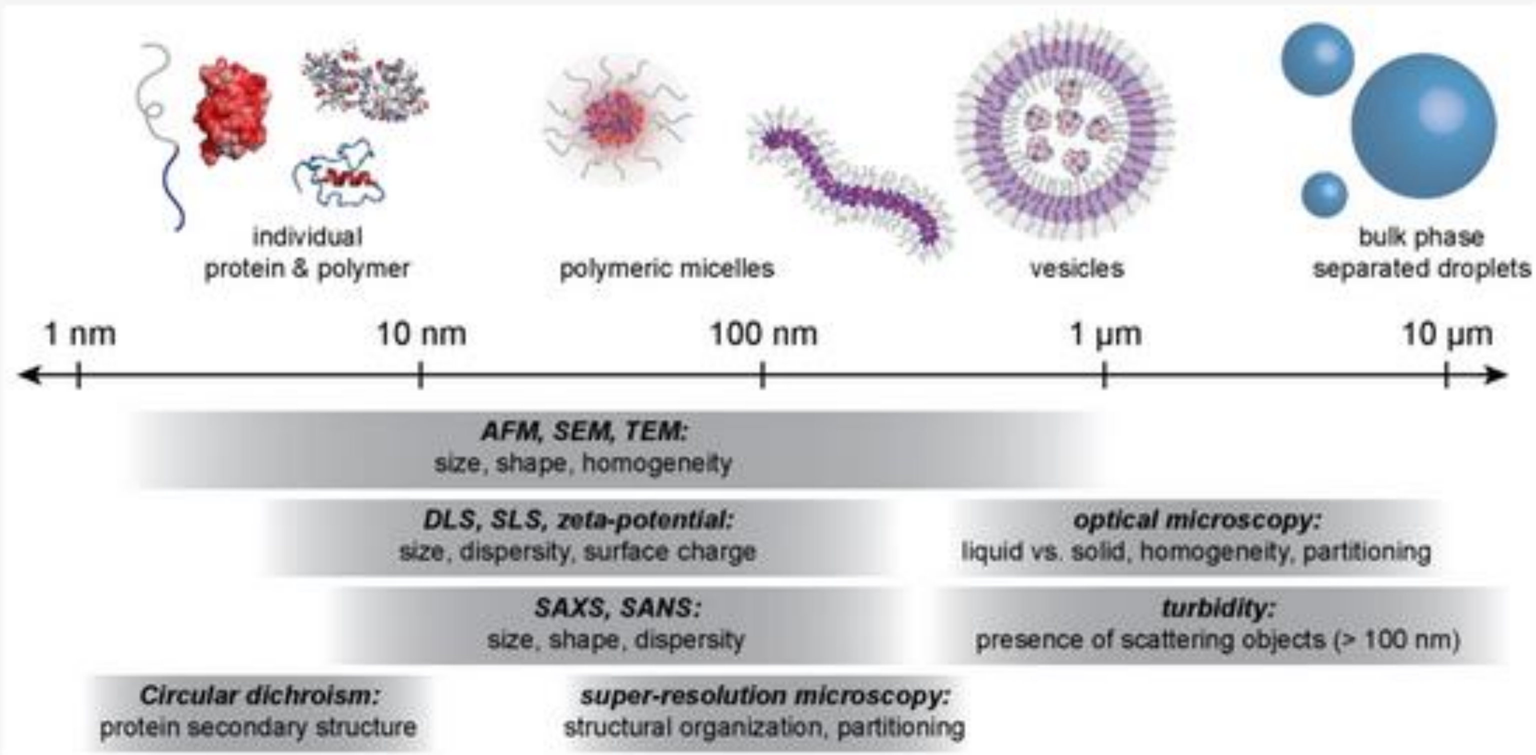
Grafting from

Grafting through



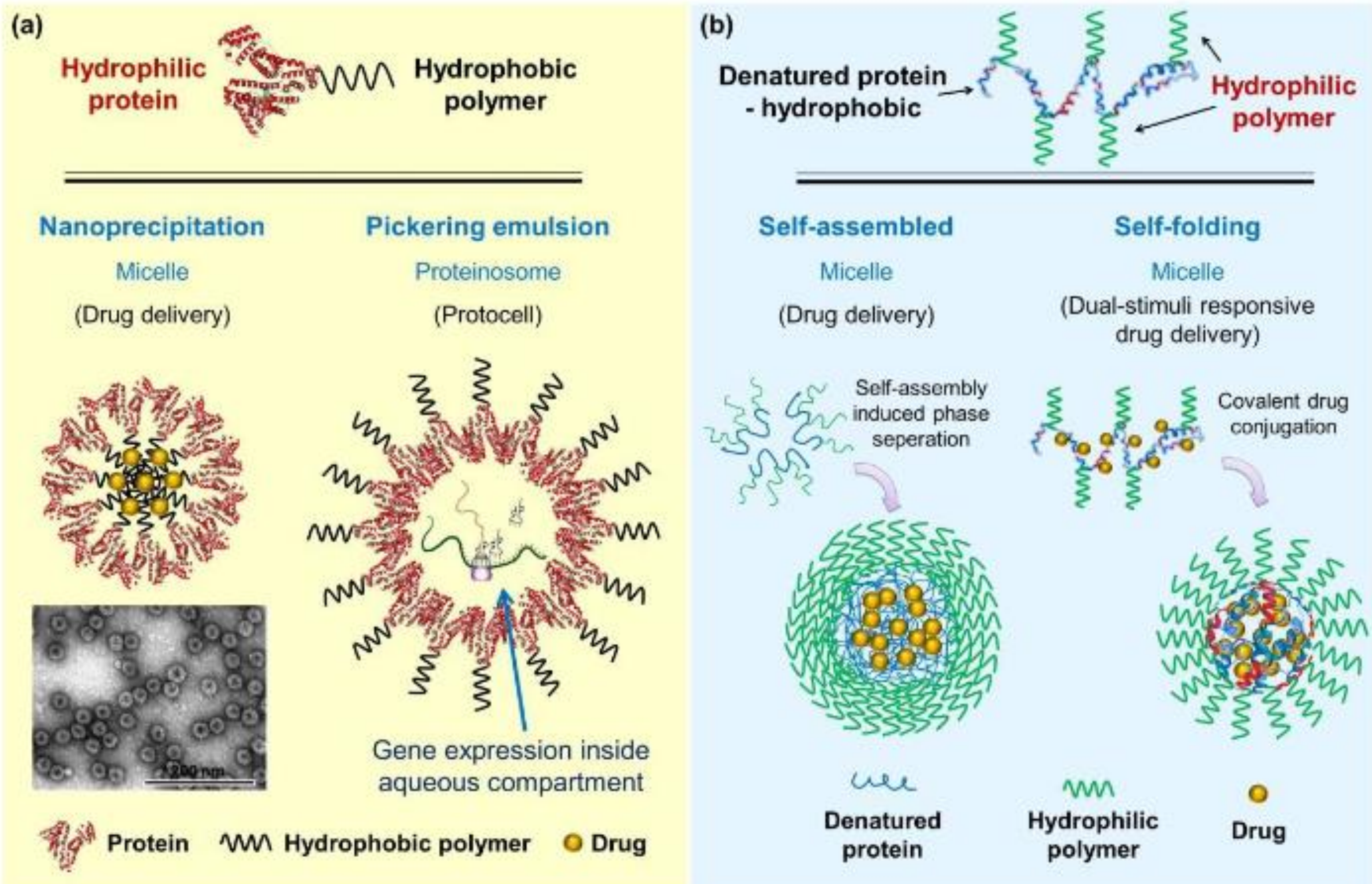
2. Supramolecular Approach to Macromolecular Self-Assembly

Figure 7. Common characterization and imaging techniques for protein–polyelectrolyte complexes. Protein–polyelectrolyte complexes span several length scales. The combination of several analytical techniques can be used to characterize the constituent molecules, micellar assemblies, and macrophase separated droplets.



DOI: 10.3390/polym11040578 (2019)

2. Supramolecular Approach to Macromolecular Self-Assembly



Biomater. Sci., 2015,**3**, 214-230

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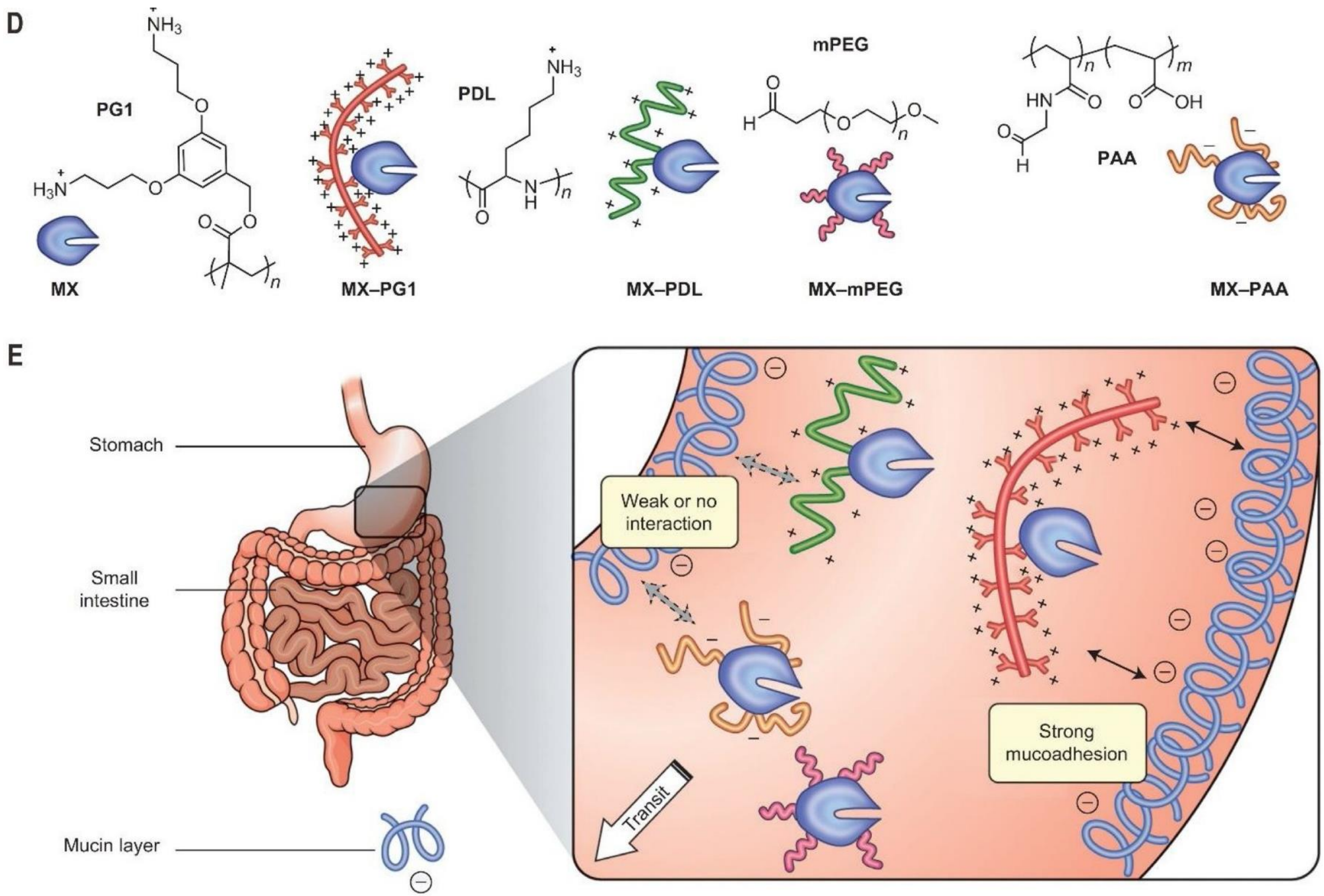


Constructing hybrid protein zymogens through protective dendritic assembly, Angew Chem Int Ed, 53 (2014), pp. 324-328
Sustained gastrointestinal activity of dendronized polymer-enzyme conjugates, Nat Chem, 5 (2013), pp. 582-589

3. Characterization of protein-polymer

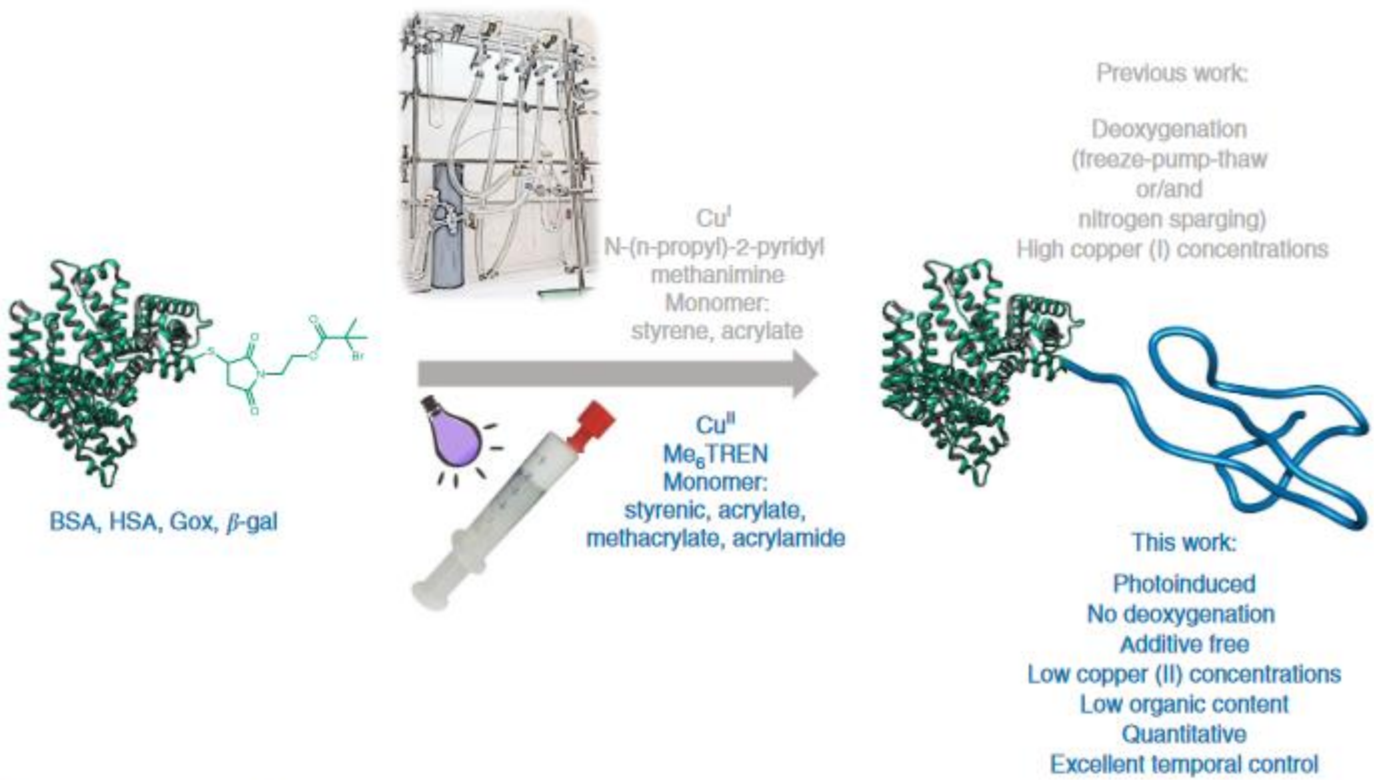


Fig. 1 General scheme and setup for the synthesis of protein-polymer amphiphiles. Top: Conventional ATRP approach and, Bottom: oxygen tolerant, photoinduced polymerization developed in this study.

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-15259-z

3. Characterization of protein-polymer

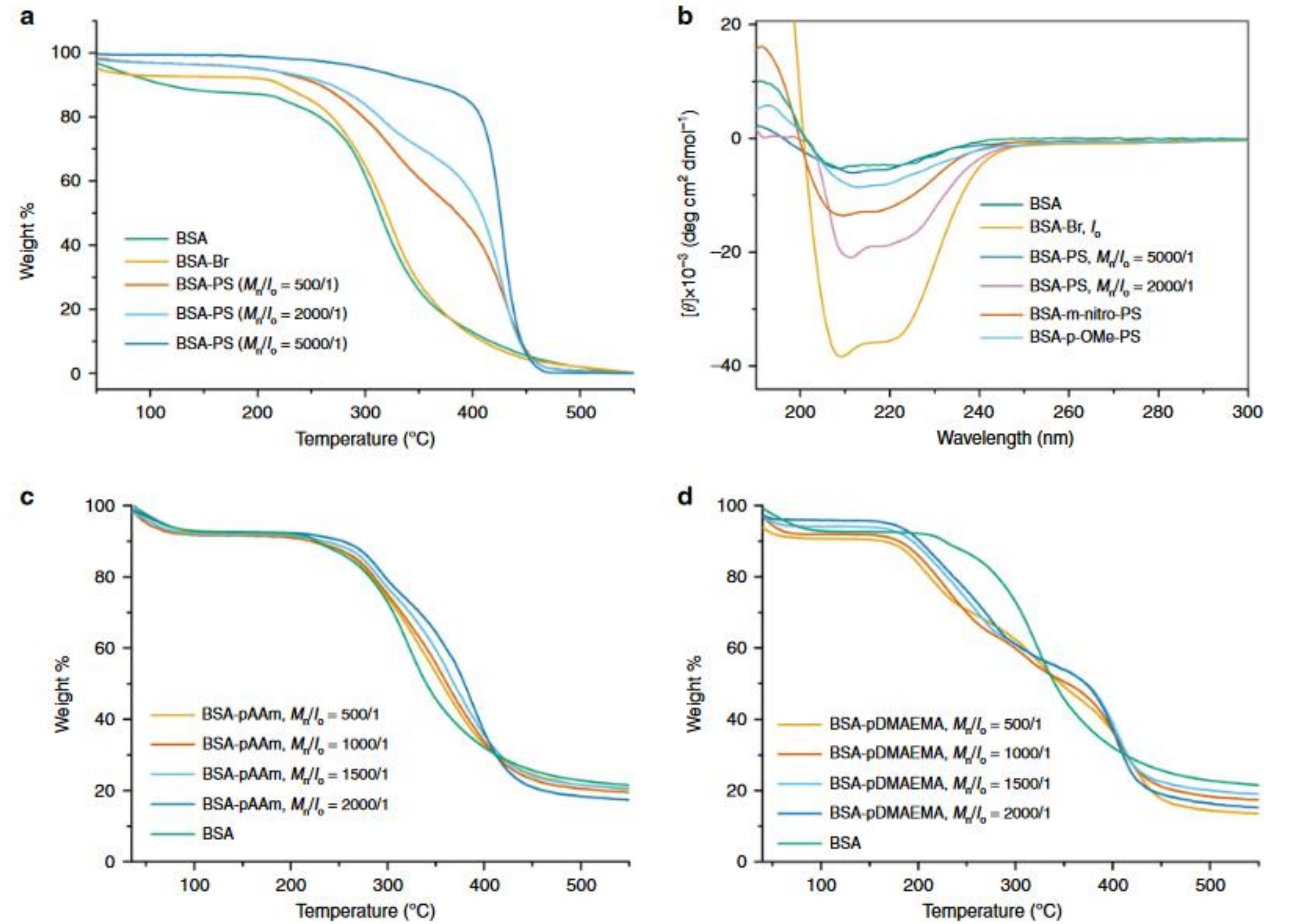


Fig. 3 Structural characterization of BSA-polymer bioconjugates. a Thermograms of BSA, BSA-Br, and BSA-PS conjugates (N_2 atmosphere). **b** CD spectra of BSA, BSA-Br (I_o), and BSA-polymer conjugates. (**c**) Thermograms of BSA, BSA-Br, and BSA-PAAm conjugates (N_2 atmosphere). **d** Thermograms of BSA, BSA-Br, and BSA-PDMAEMA conjugates (N_2 atmosphere).

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-15259-z

3. Characterization of protein-polymer

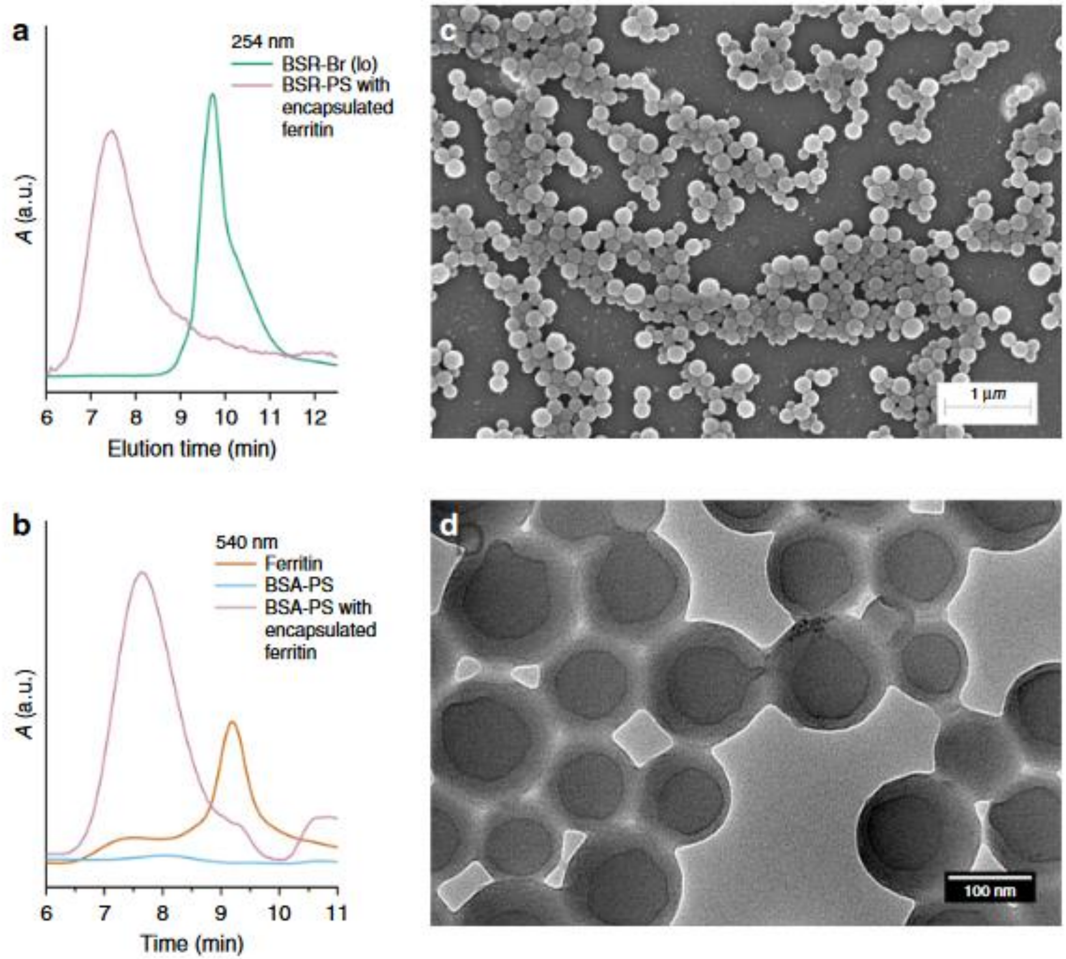
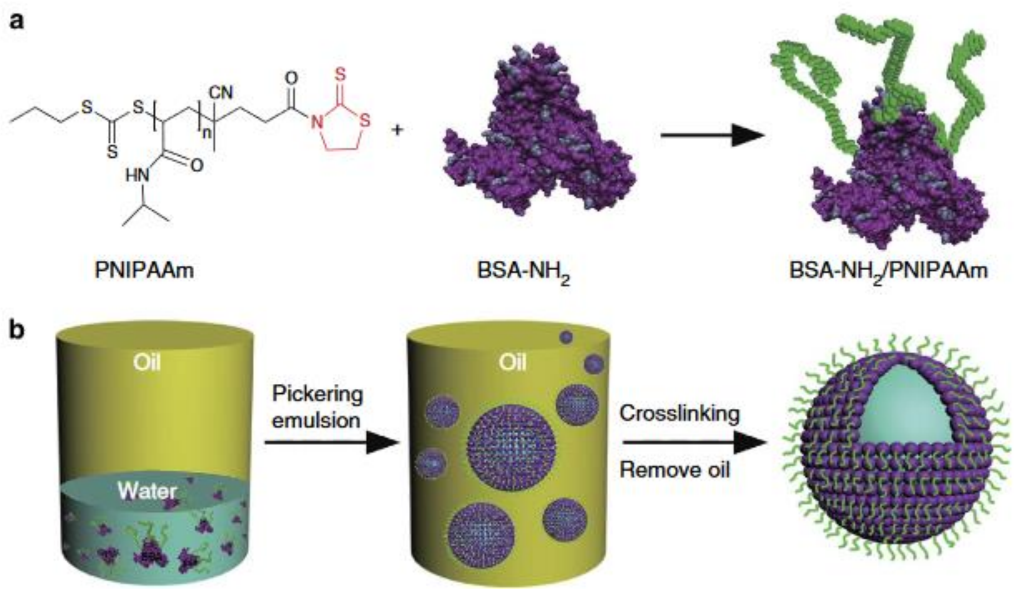


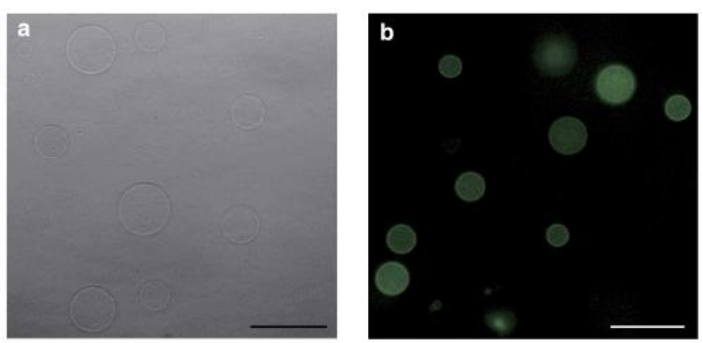
Fig. 5 BSA-PS nanocarriers—ferritin encapsulation. SEC traces at **a** 254 nm and **b** 540 nm. **c** SEM and **d** TEM micrographs of BSA-PS prepared in the presence of ferritin.

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-15259-z

3. Characterization of protein-polymer

Interfacial assembly of protein–polymer nano-conjugates into stimulus-responsive biomimetic protocells





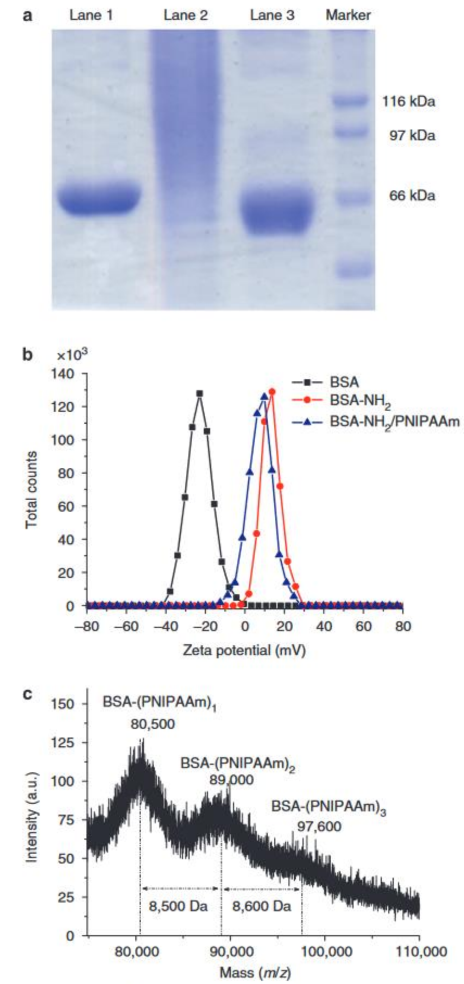
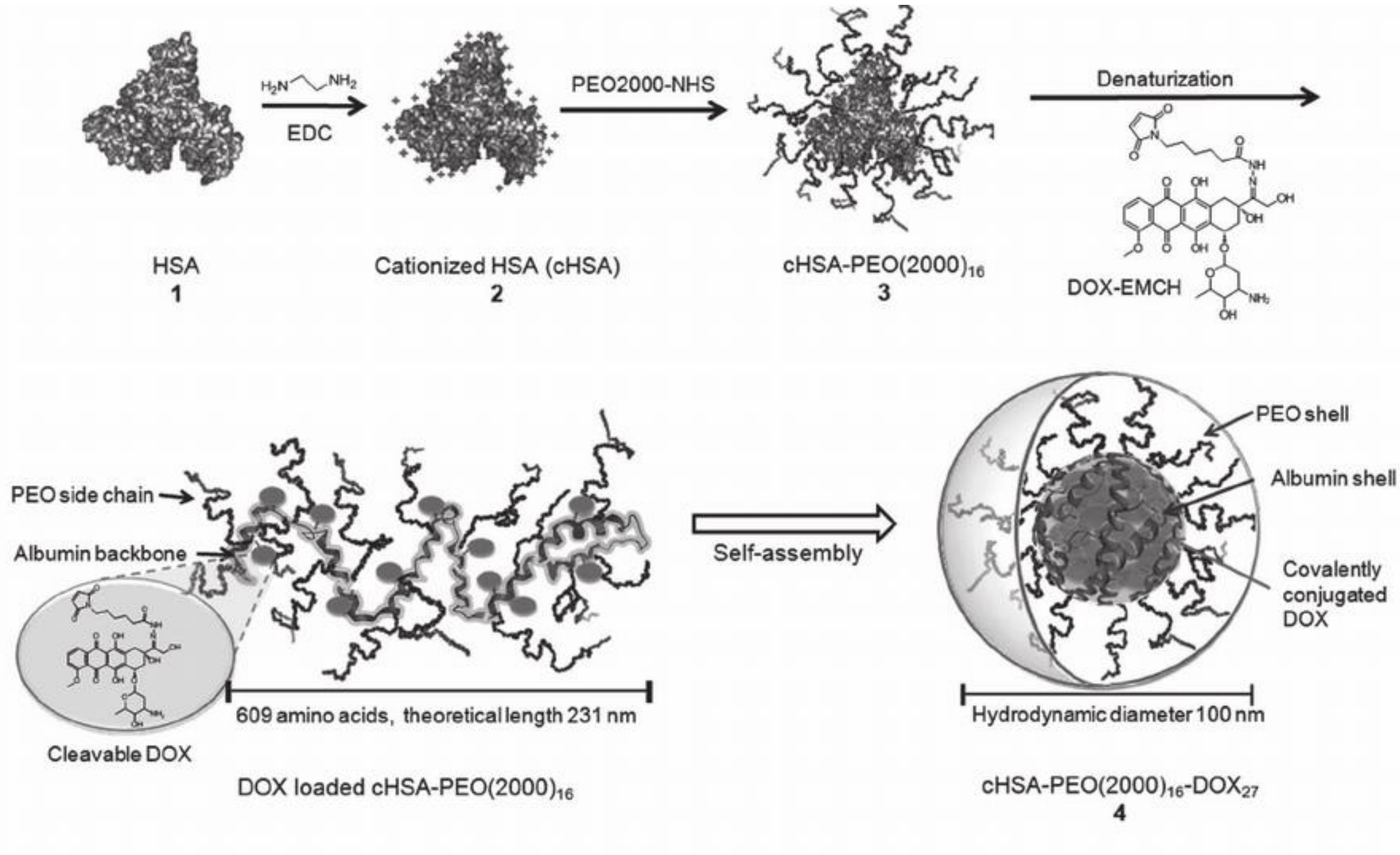


Figure 2 | Characterization of BSA-NH$_2$/PNIPAAm conjugates. **(a)** SDS-PAGE profiles; lane 1, BSA-NH$_2$; lane 2, BSA-NH$_2$/PNIPAAm; lane 3, native BSA; and marker lane. **(b)** Zeta potential measurements for BSA (black, −22 mv), BSA-NH$_2$ (red, +13 mv) and BSA-NH$_2$/PNIPAAm (blue, +9 mv) in 5.0 mM PBS pH 6.8 buffer solution at room temperature. **(c)** MALDI-TOF MS of BSA-NH$_2$/PNIPAAm conjugates showing mass peaks for BSA conjugated with one, two or three PNIPAAm chains. The mass differences between neighbouring peaks correspond to the molecular weight of the synthesized PNIPAAm (M_n 8,800 g mol^{-1}, PDI 1.19).

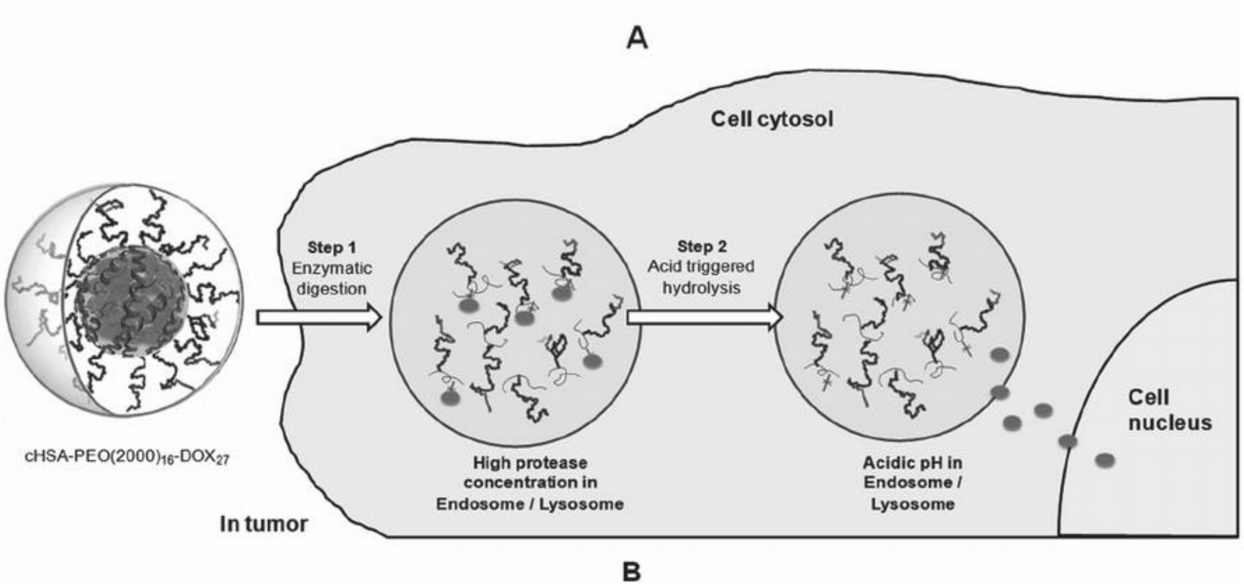
NATURE COMMUNICATIONS | 4:2239 | DOI: 10.1038/ncomms3239 www.nature.com/naturecommunications

3. Characterization of protein-polymer



Current Pharmaceutical Design, 2015, *21*, 1866-1888

3. Characterization of protein-polymer



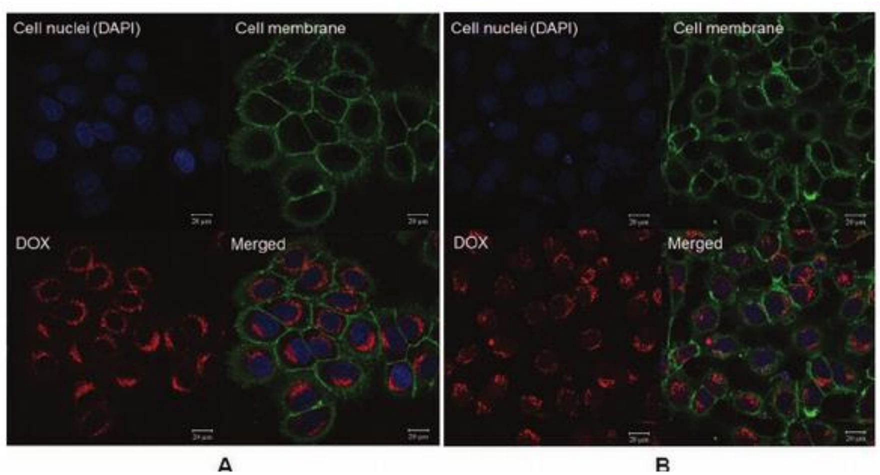


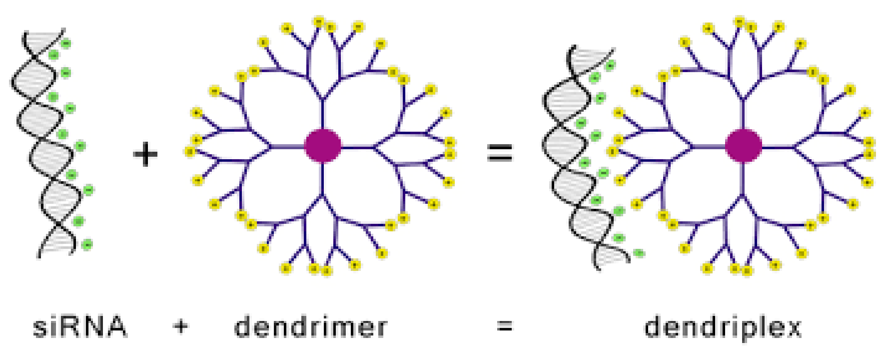
Figure 3. Confocal microscope imaging of Hela cells incubated with (A) DOX loaded cHSA-PEO(2000)$_{16}$-DOX$_{27}$ (**4**) and (B) DOX hydrochloride for 24 h.

4. Characterization of dendriplex

1. **Shape and size of dried/frozen samples** (TEM, SEM, AFM, DLS, SLS)

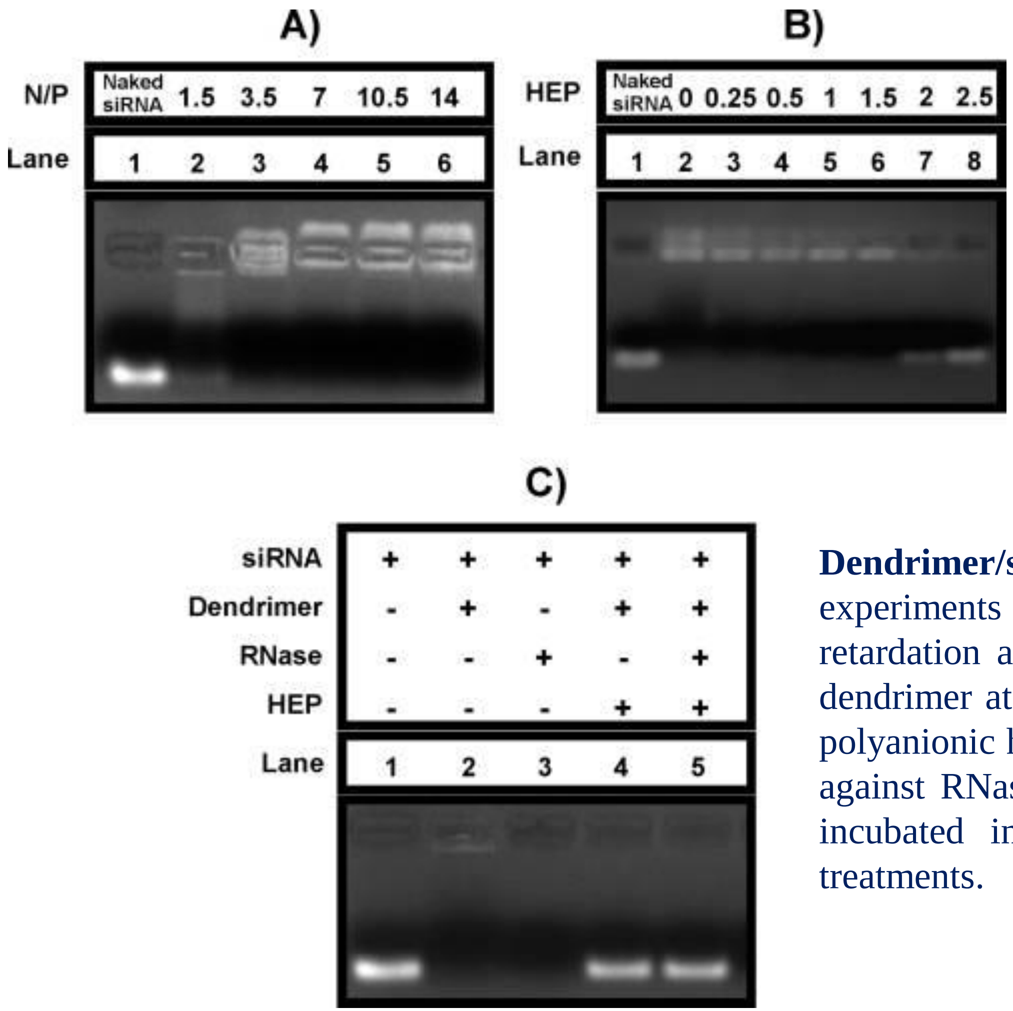
2. **Charge and molar ratio of a complex in solution** (gel electrophoresis (GE), Ethidium bromide intercalation assay (EBIA), fluorescence dye intercalation assay (FLIA), fluorescence polarization of labelled ODN (FLP), fluorescence intensity of labelled dendrimers (FL), zeta potential)

3. **Stability of dendriplexes** (Nuclease and serum protection assays, release using heparin)



Journal of Controlled Release 135 (2009) 186–197

4. Characterization of dendriplex

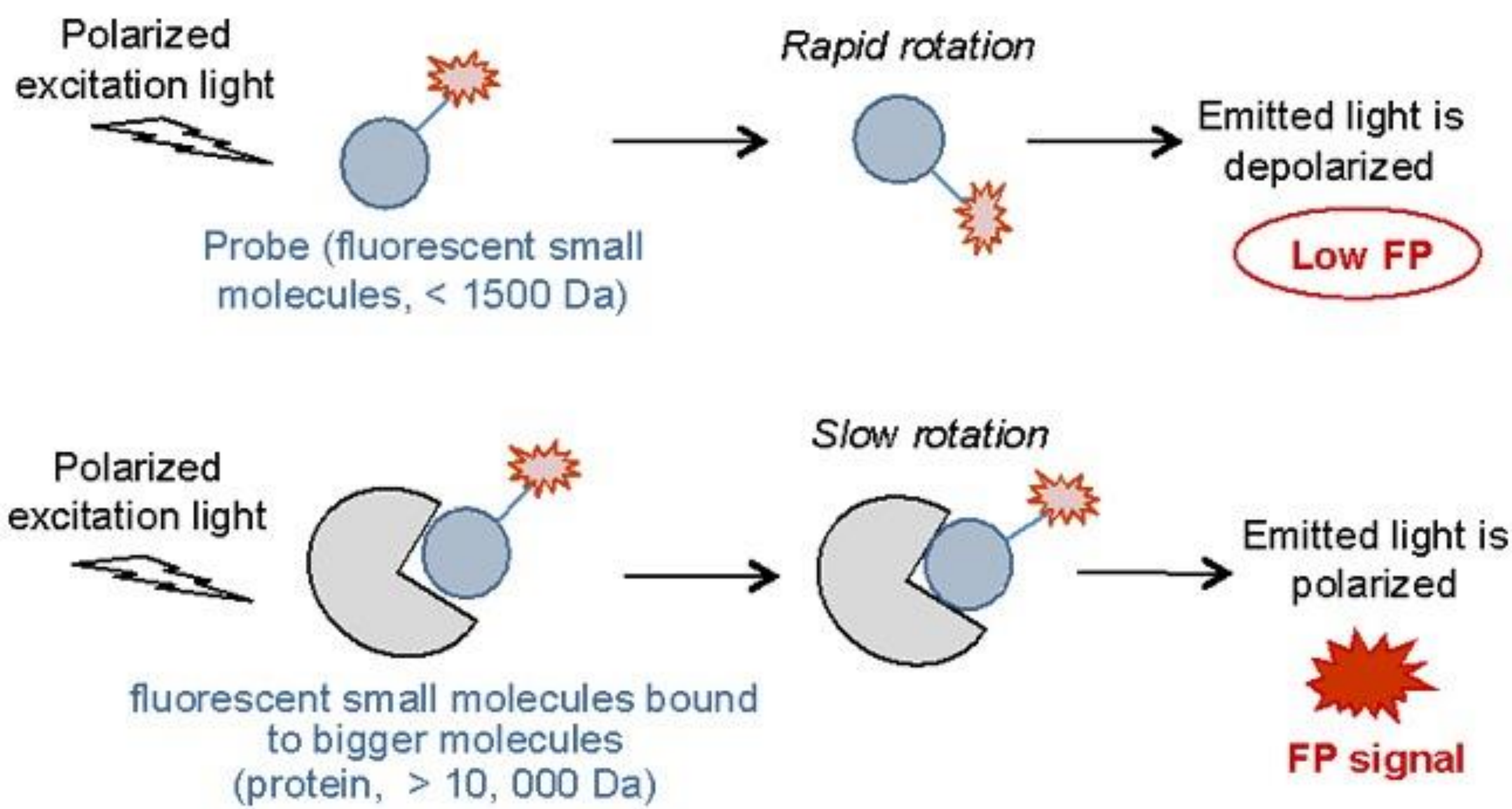


Dendrimer/siRNA dendriplex stability. Data shown for experiments repeated twice with similar results. (A) Gel retardation assay. siRNA (16.4 µg) was incubated with the dendrimer at the indicated N/P ratios. (B) siRNA release by polyanionic heparin (HEP) competition. (C) Protective effect against RNases. Dendriplexes at an N/P ratio of 10.7 were incubated in the absence or presence of the indicated treatments.

Biomacromolecules 12(4):1205-13

4. Characterization of dendriplex

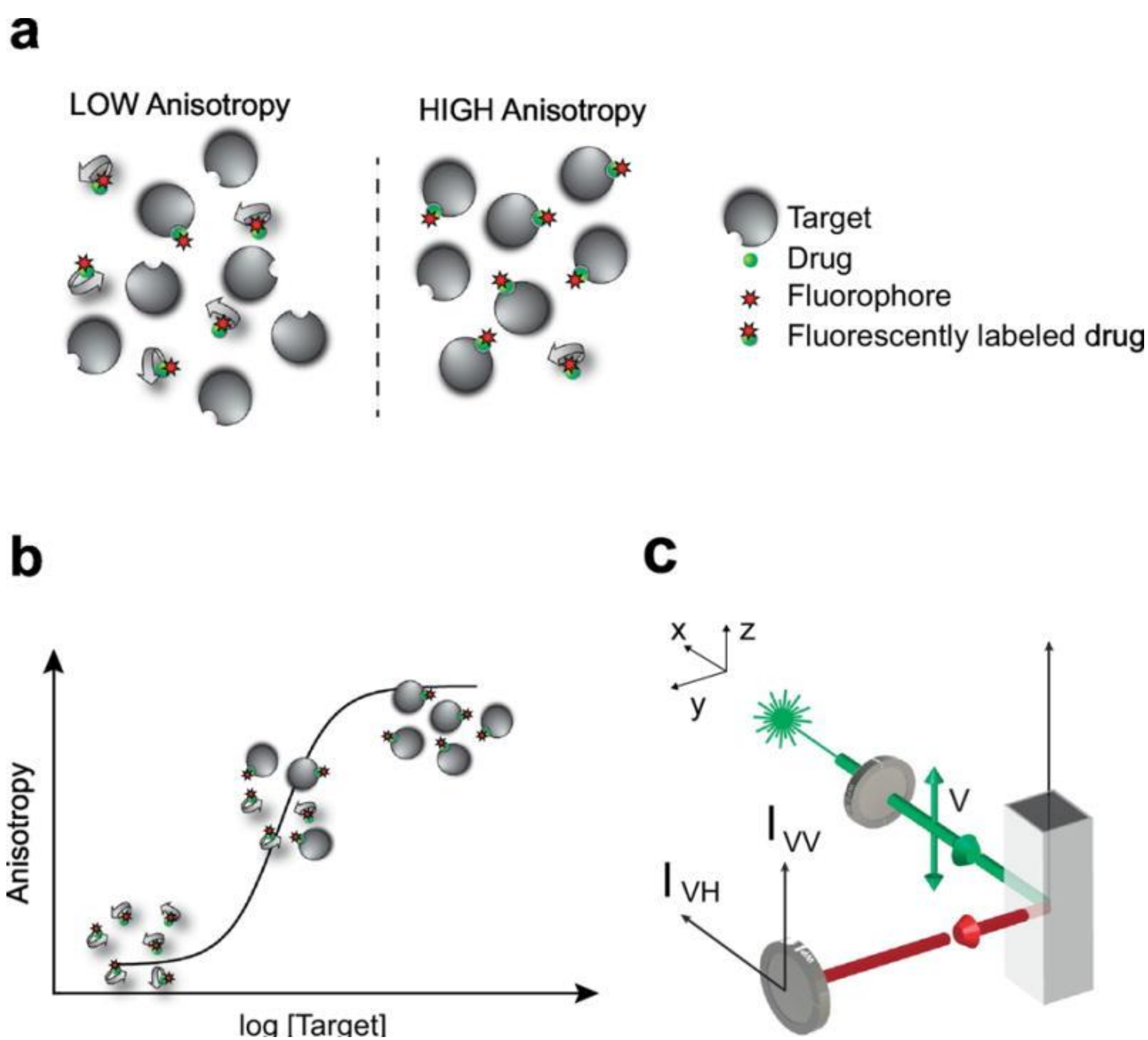
Fluorescence polarization



New in Assay Guidance Manual

4. Characterization of dendriplex

Fluorescence anisotropy



Anisotropy

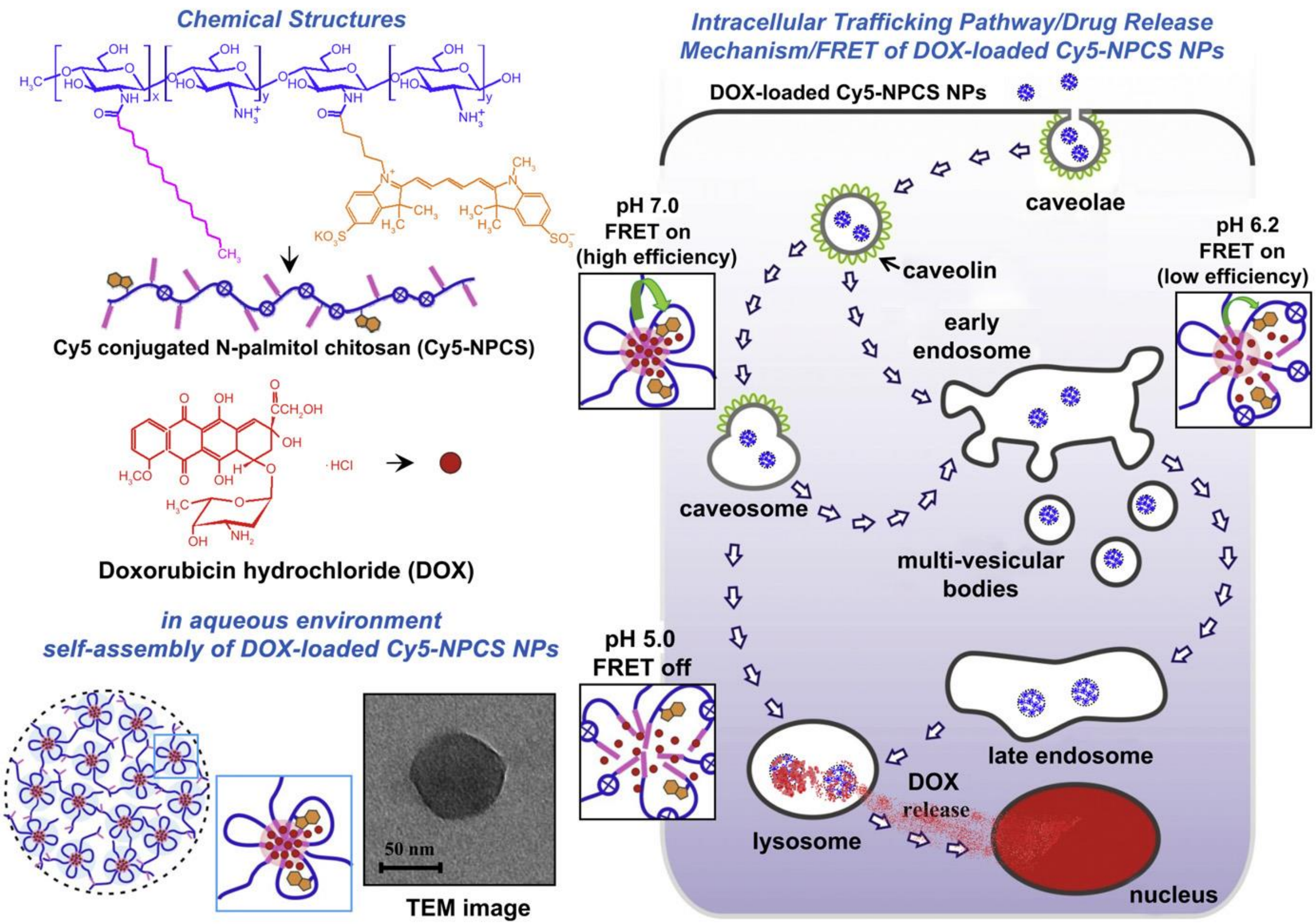
$$r = \frac{I_{VV} - G \times I_{VH}}{I_{VV} + 2G \times I_{VH}}$$

Grating Factor

$$G = \frac{I_{HV}}{I_{HH}}$$

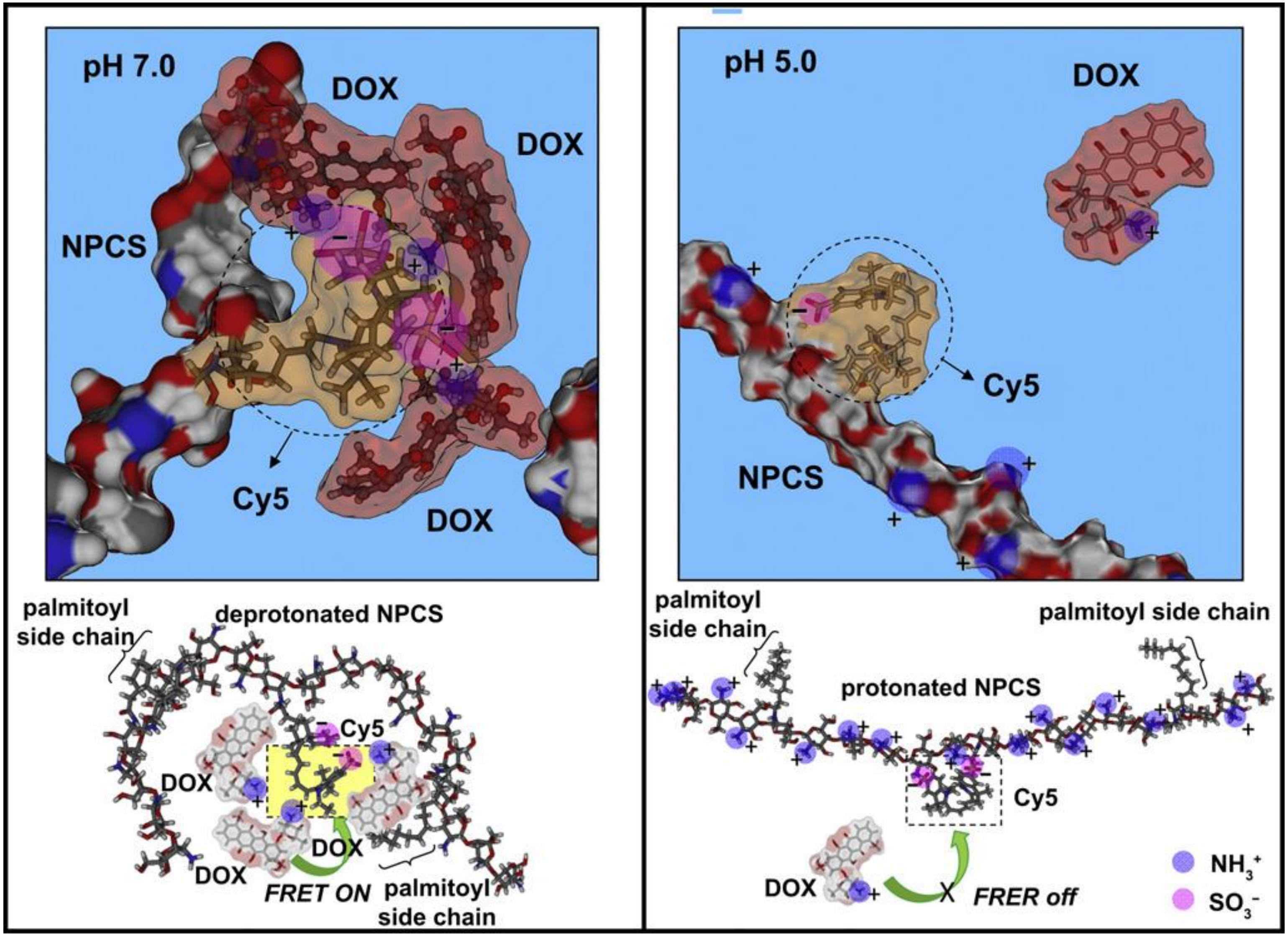
Fluorescence anisotropy imaging in drug discovery

3. Characterization of dendriplex



Biomaterials 32 (2011) 2586e2592 2587

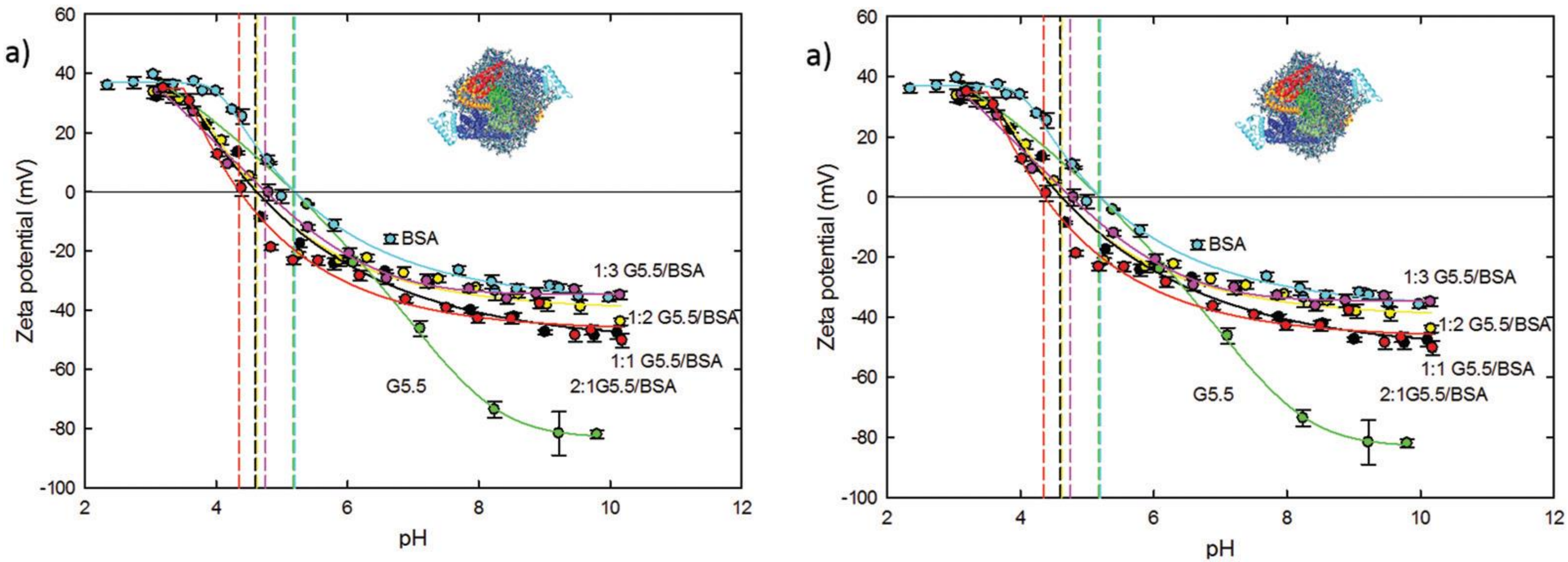
3. Characterization of dendriplex



Biomaterials 32 (2011) 2586e2592 2587

4. Characterization of protein-dendrimer interaction

Analysis of dendrimer-protein interactions and their implications on potential applications of dendrimers in nanomedicine



(a) Dependence of zeta potential on pH for G5.5 PAMAM/BSA complexes with varied molar ratios. (b) Comparison influence of pH of complex formation on zeta potential.

Nanoscale, 2021,**13**, 2703-2713

4. Characterization of protein-dendrimer interaction

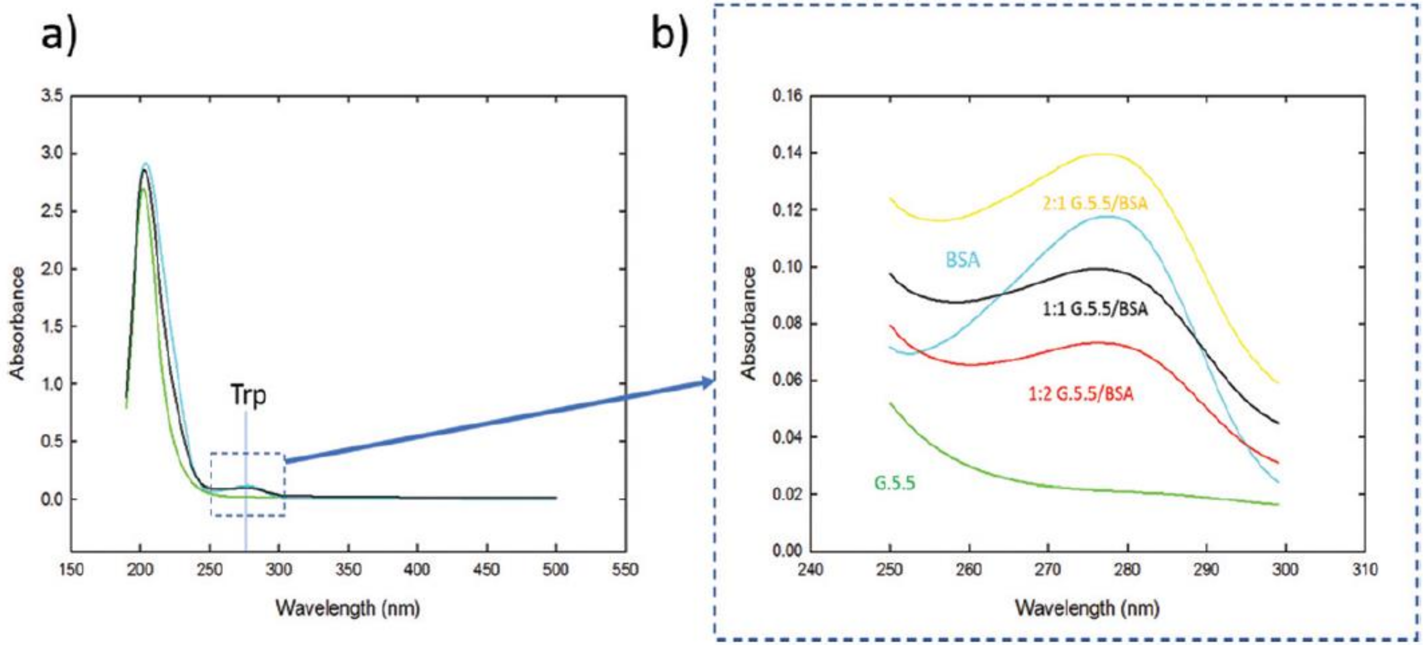


Fig. 6 (a) UV-vis spectra G5.5 + BSA indicating Trp position. (b) Comparison of UV-vis spectra for varying compositions of G5.5/BSA complexes.

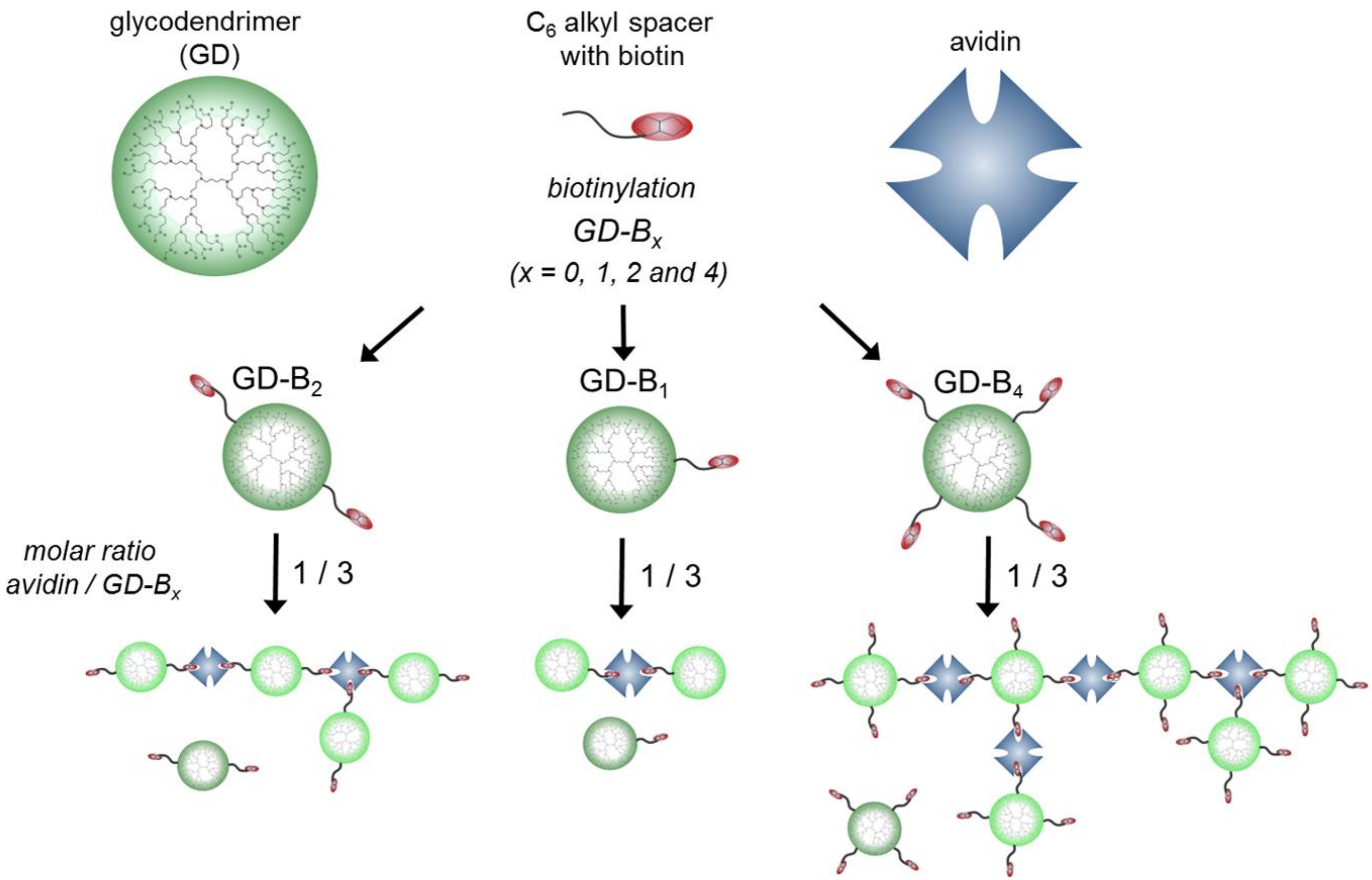
Table 3 Comparison of characteristics of G5.5/BSA complexes formed

Ratio G5.5 : BSA	2 : 1	1 : 1	1 : 2	1 : 3
Effective ratio G5.5 : BSA (from UV-vis)	1 : 0.25	1 : 0.47	1 : 0.73	1 : 1.68
Zeta potential (ζ)	−41 mV	−38 mV	−33.4 mV	−32 mV
DLS R_H	Aggregates	4.06 nm	4.15 nm	3.95 nm

Nanoscale, 2021,**13**, 2703-2713

5. Characterization of Biohybrid structures

From 1D Rods to 3D Networks: A Biohybrid Topological Diversity Investigated by Asymmetrical Flow Field-Flow Fractionation



Dealing with the complexity of conjugated and self-assembled polymer-nanostructures using field-flow fractionation Anal Sci Adv. 2021;2:95–108.

Macromolecules 2015, 48, 4607–4619

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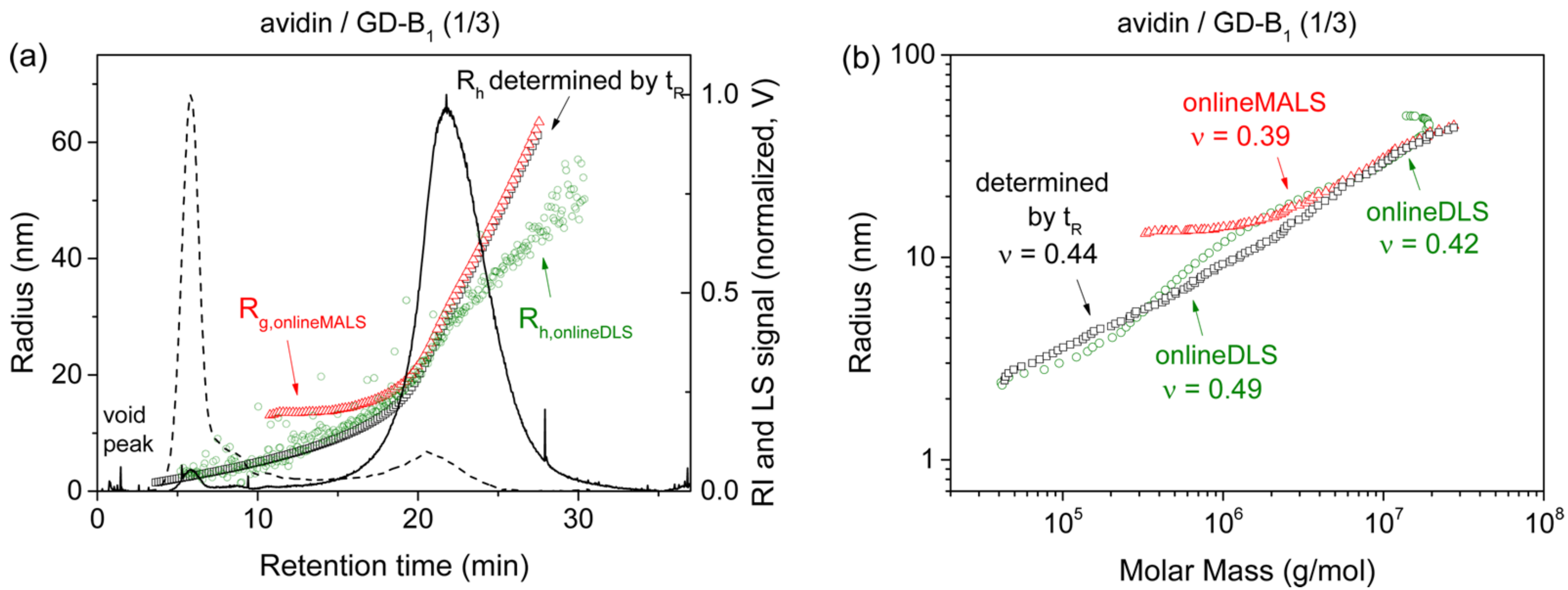

(a) AF4 fractograms (RI signal, dashed line; LS signal, solid line) with differently determined radii by online MALS (Rg, red triangles), by online DLS (Rh, green circles), and by retention times (Rh, black squares) and (b) conformation plot with differently determined radii as a function of molar mass with calculated scaling factors of biohybrid structures formed by avidin/GD-B1 (1/3).

Macromolecules 2015, 48, 4607–4619

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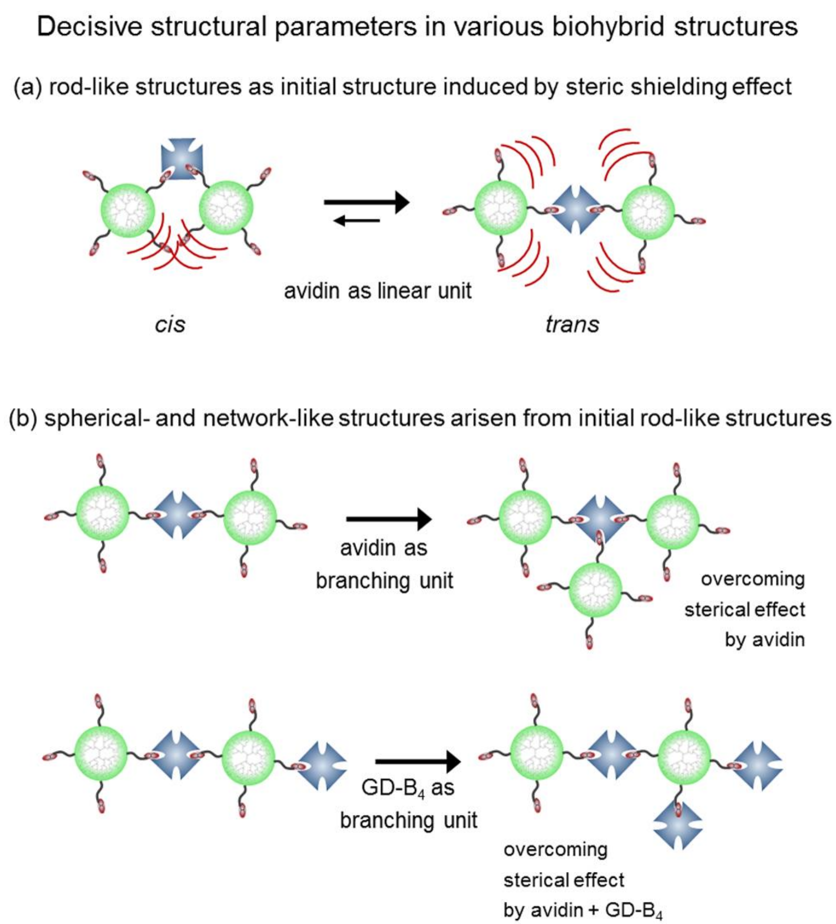
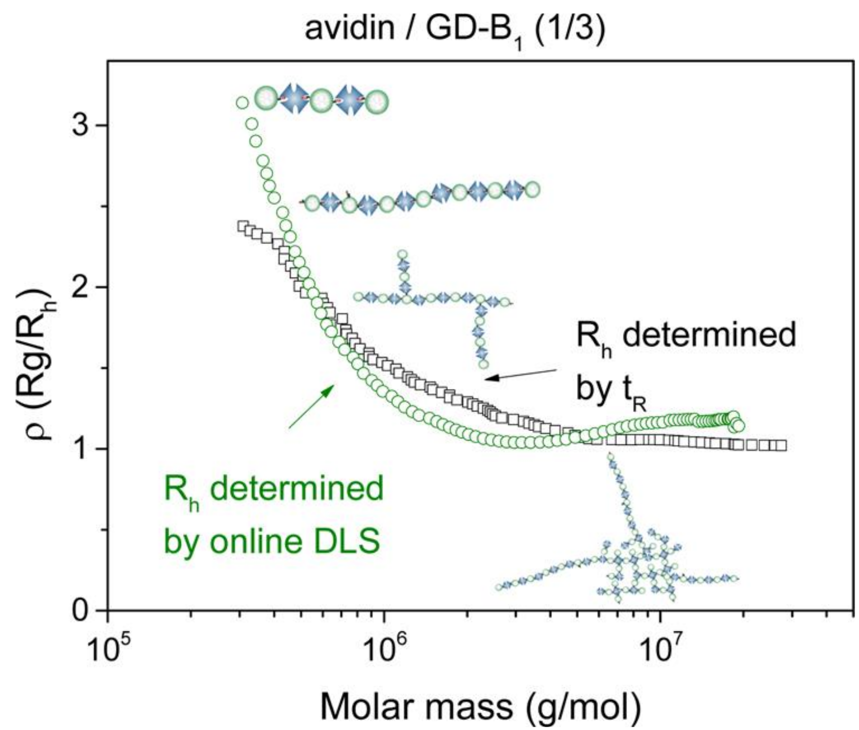
From 1D Rods to 3D Networks: A Biohybrid Topological Diversity Investigated by Asymmetrical Flow Field-Flow Fractionation





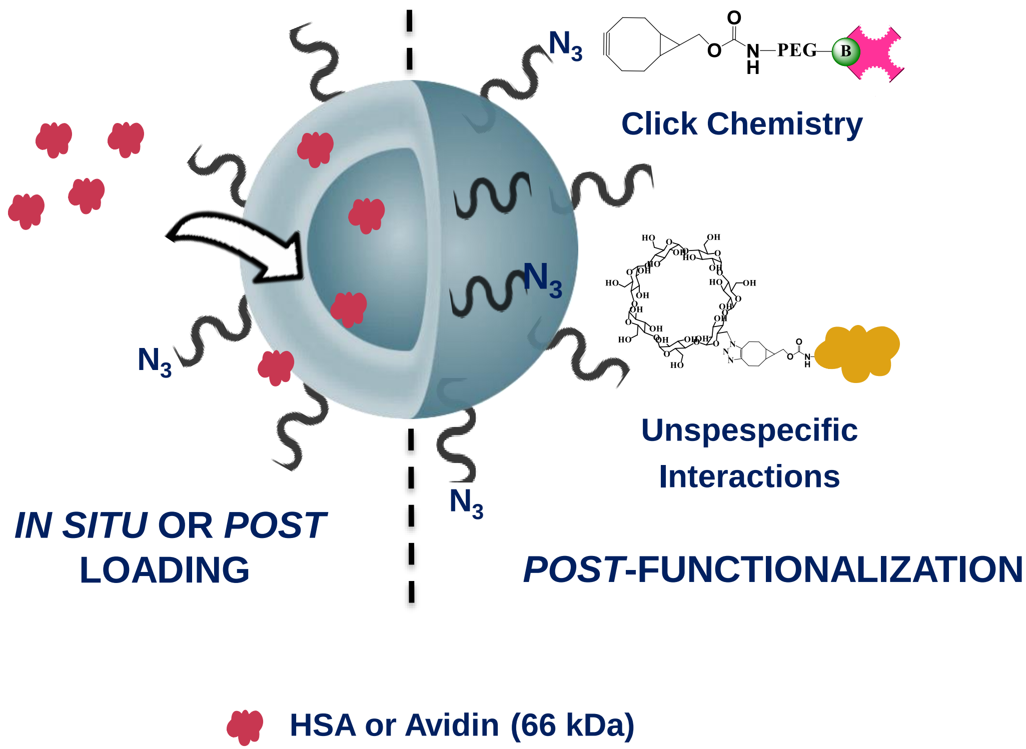
ρ parameter (Rg/Rh) vs molar mass, comparison of Rh determination by retention times (black squares) and by online DLS (green circles) of biohybrid structures formed by avidin/GD-B1 (1/3).

Macromolecules 2015, 48, 4607−4619

5. Characterization of Biohybrid structures

Multivalent Protein-Loaded pH-Stable Polymersomes: First Step Towards Protein Targeted Therapeutics

Multivalent Protein-Loaded Polymersomes



Specific coupling ➔ Faster Cellular Uptake

S. Moreno *et al*, *Macromol. Biosci.* **2021**, 2100102

5. Characterization of Biohybrid structures

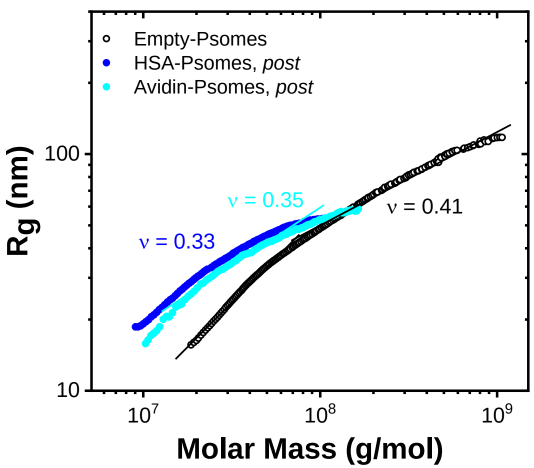
Multivalent Protein-Loaded pH-Stable Polymersomes: First Step Towards Protein Targeted Therapeutics

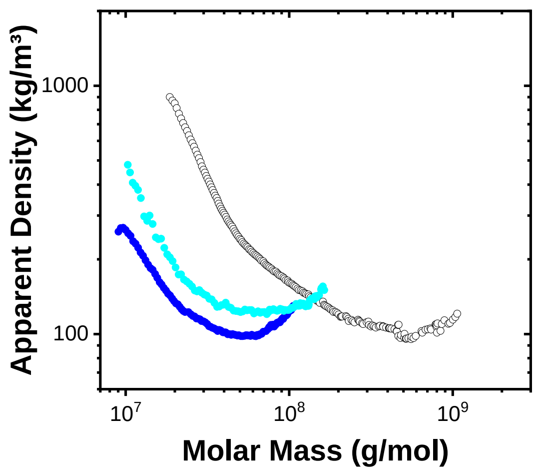
Protein-loaded polymersomes (Avidin- and HSA-Psomes) by **POST Loading**

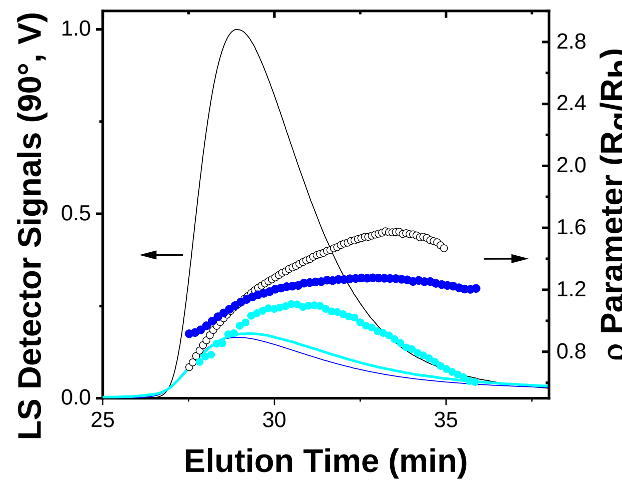
ν scaling parameter - slope
ν = 0.33 – ideal spherical shape
ν strongly depends on surface + membrane composition!

Decrease of apparent density
Larger R_g + incorporation into the membrane

ρ parameter (R_g/R_h)
ρ ~ 1 hollow sphere capsule (HSA-Psomes)
ρ ~ 0.88 hard/filled sphere (Avidin-Psomes)





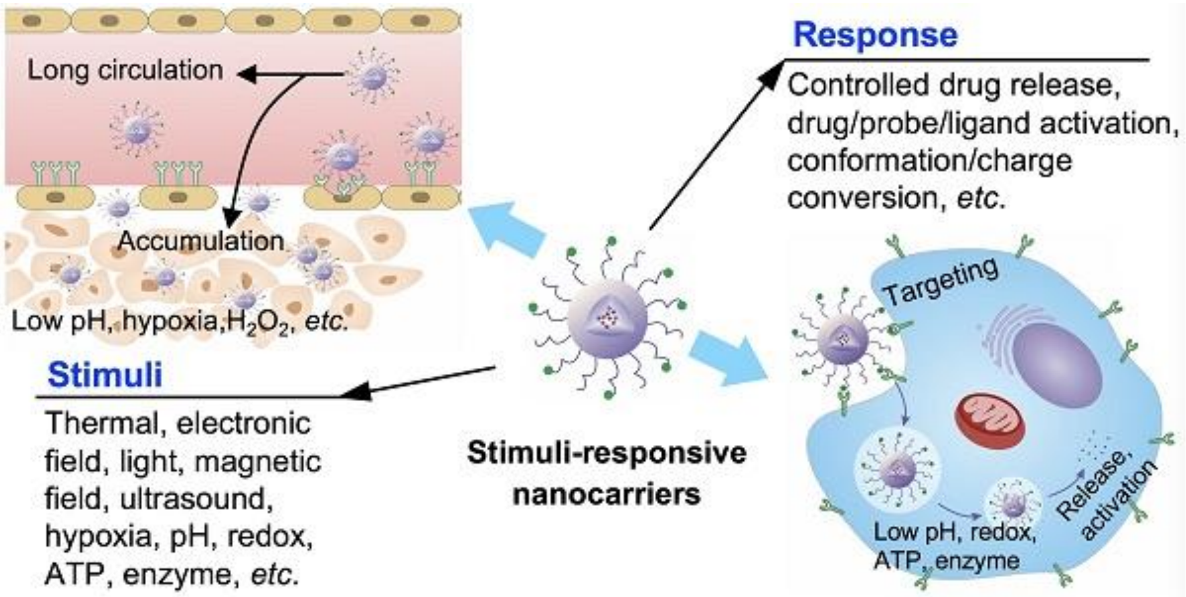


Cooperation

Prof. Dr. Albena Lederer and Dr. Susanne Boye (IPF Dresden)

S. Moreno *et al*, *Macromol. Biosci.* **2021**, 2100102

6. Self assembled nanoparticles for biomedical application



Nanoparticles also can act as a "medium and carrier"

- *(i)* *Size and flexibility,* the small and controllable size is suitable for conducting antimicrobial operations;
- *(ii)* *Protection*, drugs are protected from detrimental chemical reactions improving the potency of the drugs;
- *(iii)* *Precision and security*, nanocarriers help to target antibiotics to an infection site minimizing systemic side effects;
- *(iv)* *Controllability,* sustained and controllable release of antibiotics can be achieved flexibly;
- *(v)* *Combination or synergic effects*, multiple drugs or antimicrobials can be packaged within the same nanocarrier.