Targeted Polymeric Nanoparticles ~drug delivery~

Literature Seminar

May 21, 2012

Soichi Ito (M1)

Contents

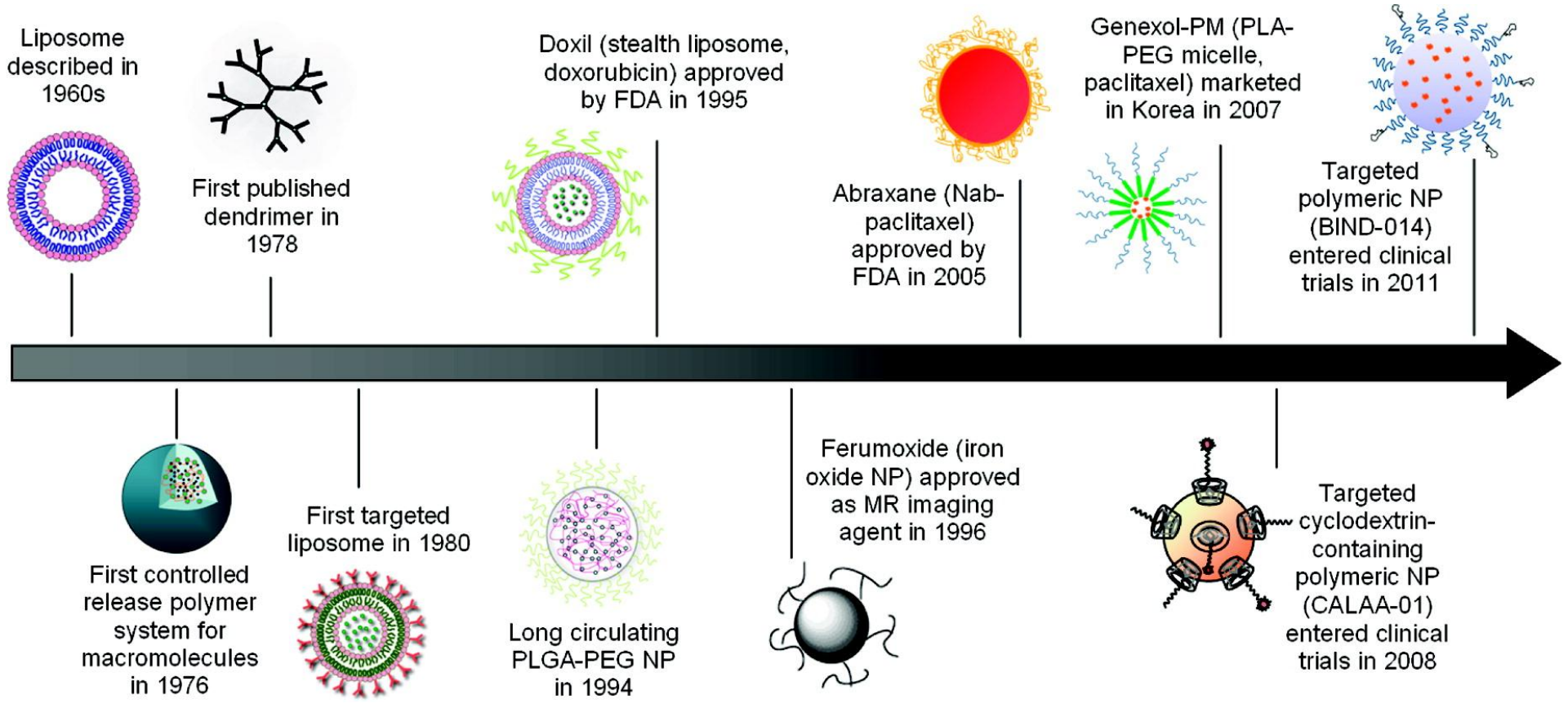
I. Introduction

II. Topics

1. Passive *vs* Active targeting
2. Preparation of targeted polymeric NPs
3. Targeting ligands
4. Optimal biophysicochemical characteristics

III. Perspective

Historical timeline of clinical-stage nanoparticle technologies

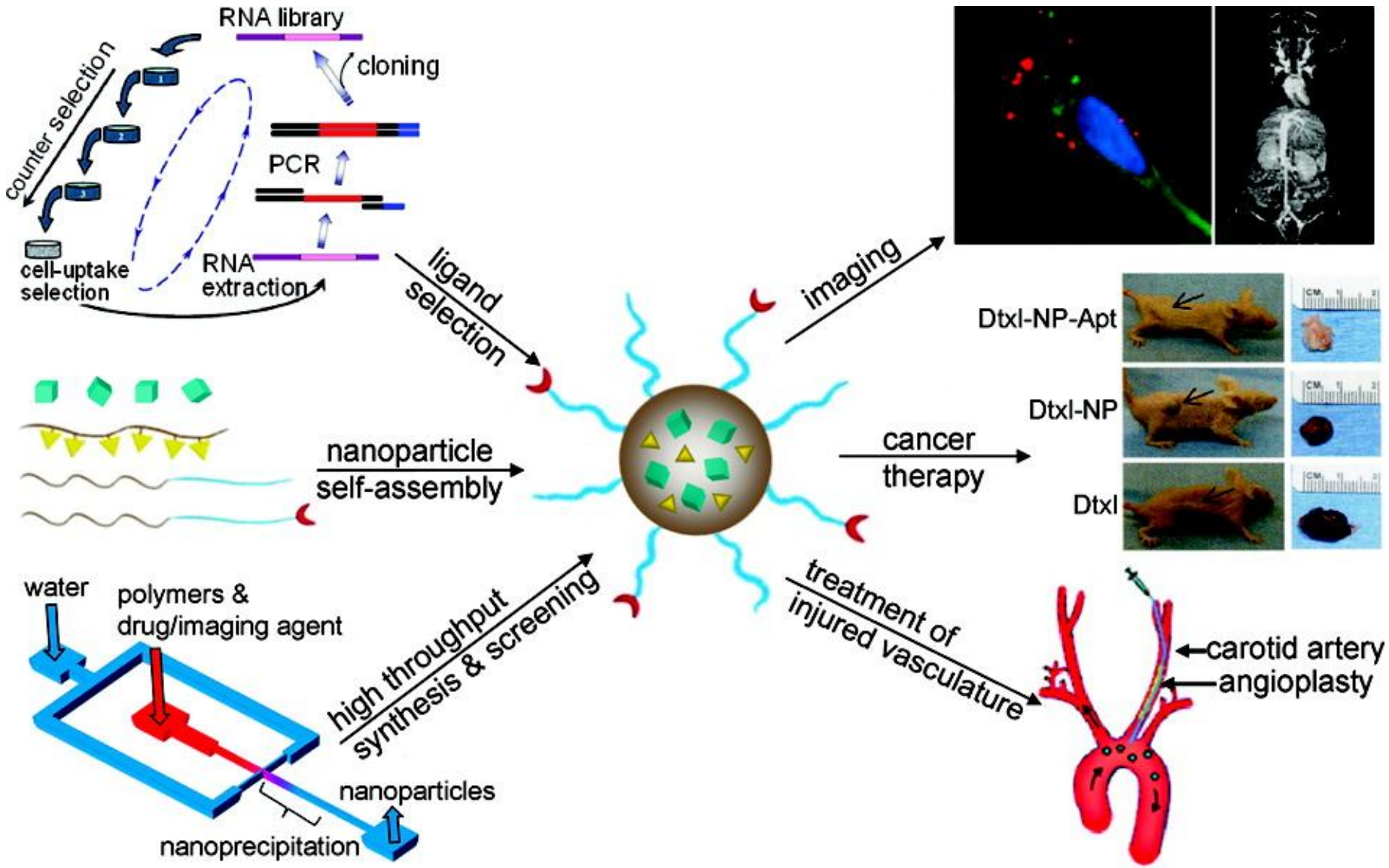


JINJUN SHI *et al*. *ACCOUNTS OF CHEMICAL RESEARCH*, 2011, *44(10),* 1123–1134.

- Polymeric NPs have the capability to

1. release drugs at an experimentally predetermined rate over a prolonged period of time,
2. release drugs preferentially at target sites with the possibility of controlled release rates,
3. maintain drug concentrations within therapeutically appropriate ranges in circulation and within tissues,
4. protect drugs from hepatic inactivation, enzymatic degradation and rapid clearance *in vivo*.

Targeted polymeric NPs



JINJUN SHI *et al*. *ACCOUNTS OF CHEMICAL RESEARCH*, 2011, *44(10),* 1123–1134.

Targeted NPs in clinical development

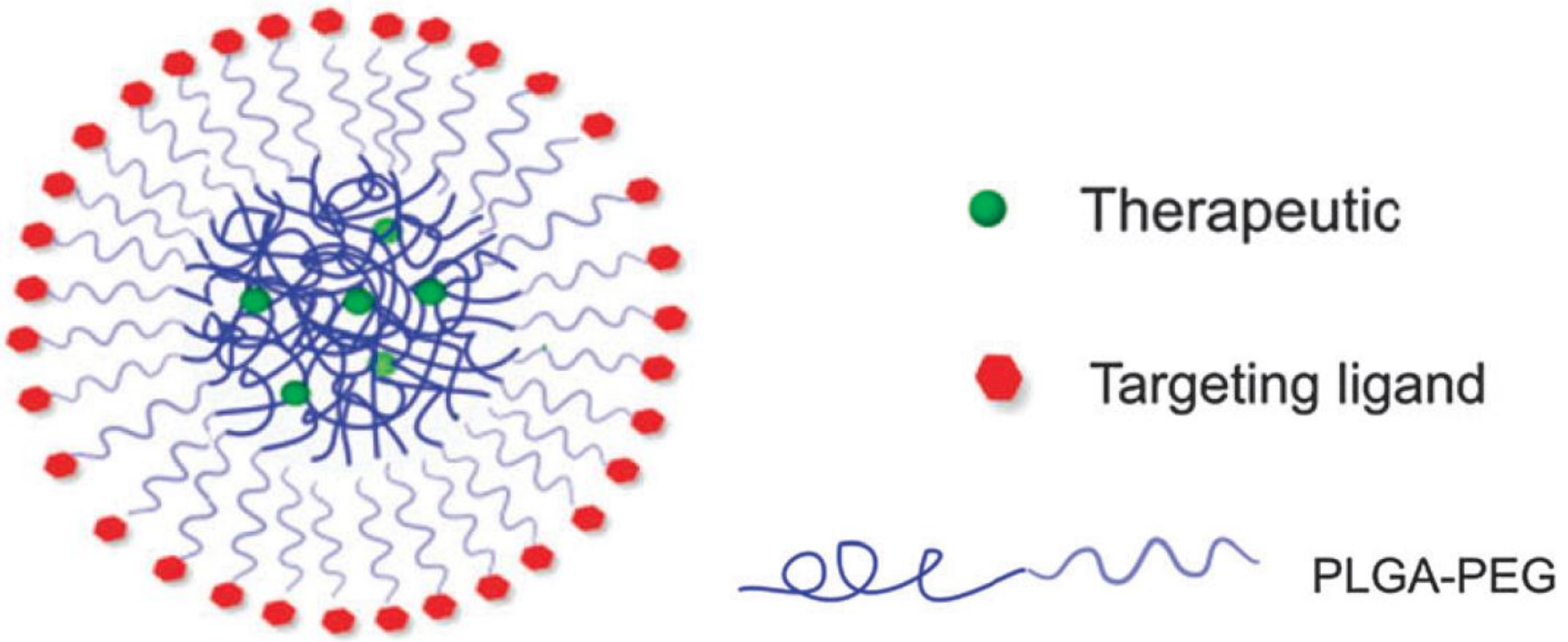
Table 1 Targeted NPs in clinical development

Identity	Ligand	Target	Nanoparticle	Active Pharmaceutical Ingredient (API)
BIND-014	Small molecule	PSMA[a]	Polymeric	Docetaxel
SEL-068	Small molecule	Antigen presenting cells	Polymeric	Nicotine antigen T-helper cell peptide, TLR[b] agonist
CALAA-01	Transferrin	Transferrin receptor	Polymeric	siRNA
MBP-426	Transferrin	Transferrin receptor	Liposome	Oxaliplatin
MCC-465	Antibody fragment	Tumour antigen	Liposome	Doxorubicin
SGT53-01	Antibody fragment	Transferrin receptor	Liposome	p53 gene

[a] PSMA: prostate specific membrane antigen. [b] TLR: Toll-Like Receptor agonist.

Nazila Kamaly *et al. Chem. Soc. Rev.,* 2012, *41*, 2971–3010

What is the targeted polymeric NPs?

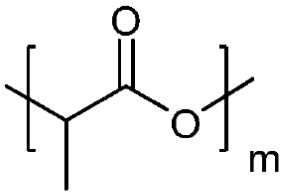


poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG)

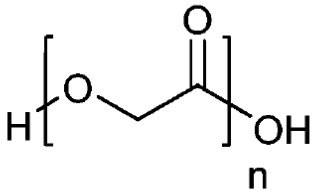
Nazila Kamaly *et al*. *Chem. Soc. Rev.,* 2012, *41*, 2971–3010.

Biodegradable polymers

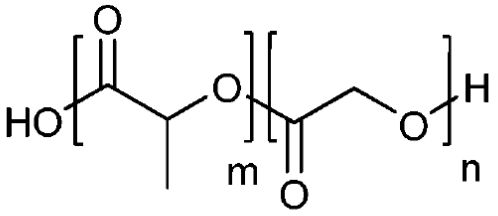
"Controlled Drug Release"



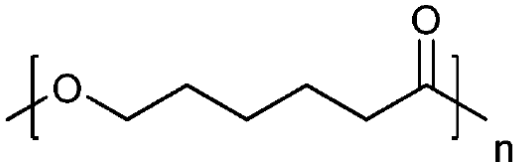
PLA



PGA



PLGA

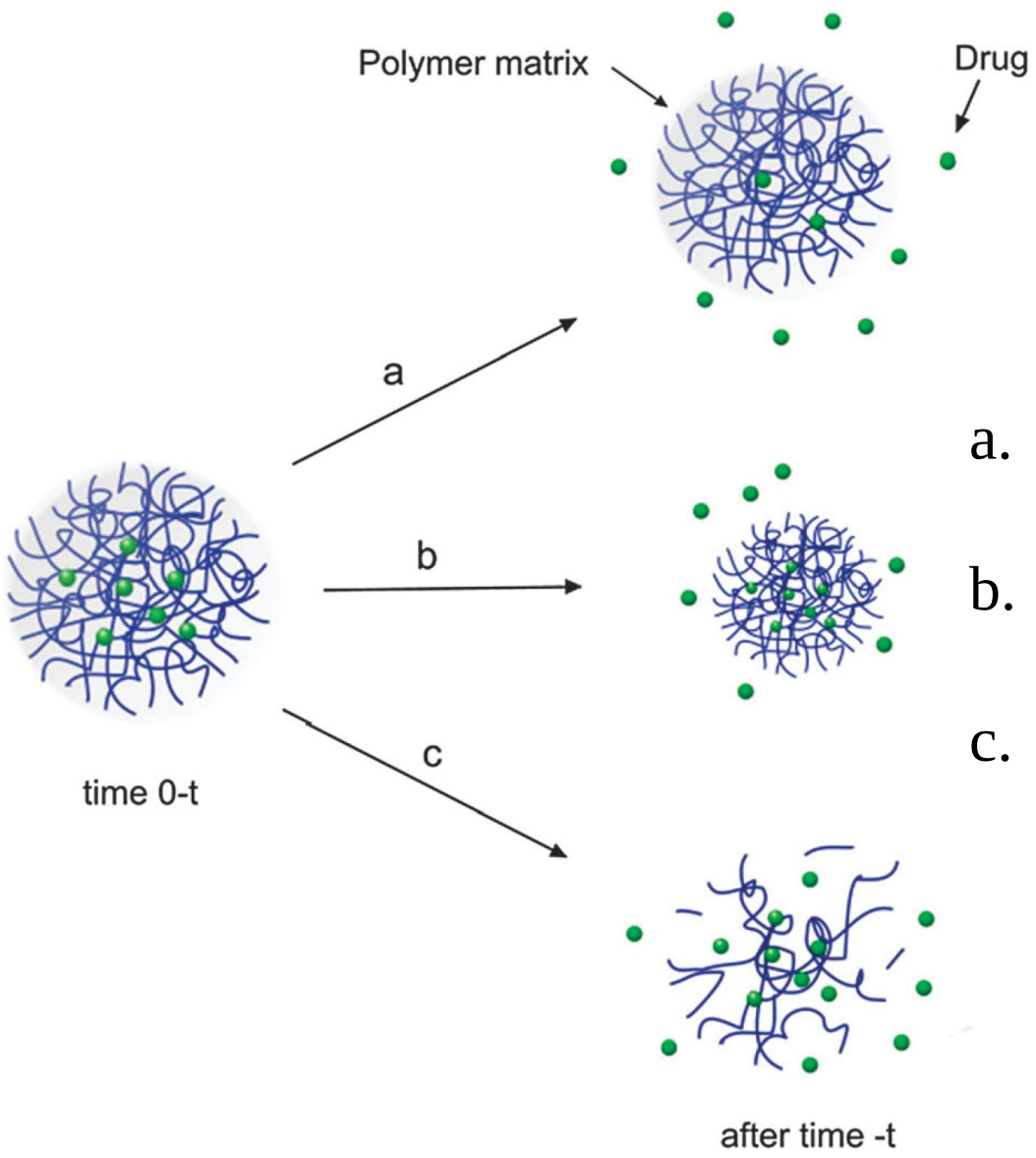


PCL

poly(lactic acid) (PLA), poly(glycolic acid) (PGA),
poly(lactic-co-glycolic acid) (PLGA), poly(caprolactone) (PCL)

Nazila Kamaly *et al*. *Chem. Soc. Rev.,* 2012, *41*, 2971–3010.

Drug release mechanisms



a. Diffusion from polymer matrix
b. Surface erosion/degradation of polymer matrix
c. Biodegradation of polymer matrix due to hydrolytic degradation

Nazila Kamaly *et al*. *Chem. Soc. Rev.,* 2012, *41*, 2971–3010.

“Stealth” Nanopartile

- ✓ The non-specific binding of plasma proteins onto the surface of NPs, also known as opsonization, leads to enhanced blood clearance by the cells of mononulear phagocytic system (**MPS**).

- ➢ By decorating the surfaces of NPs with PEG polymers, the circulation times can be prolonged.

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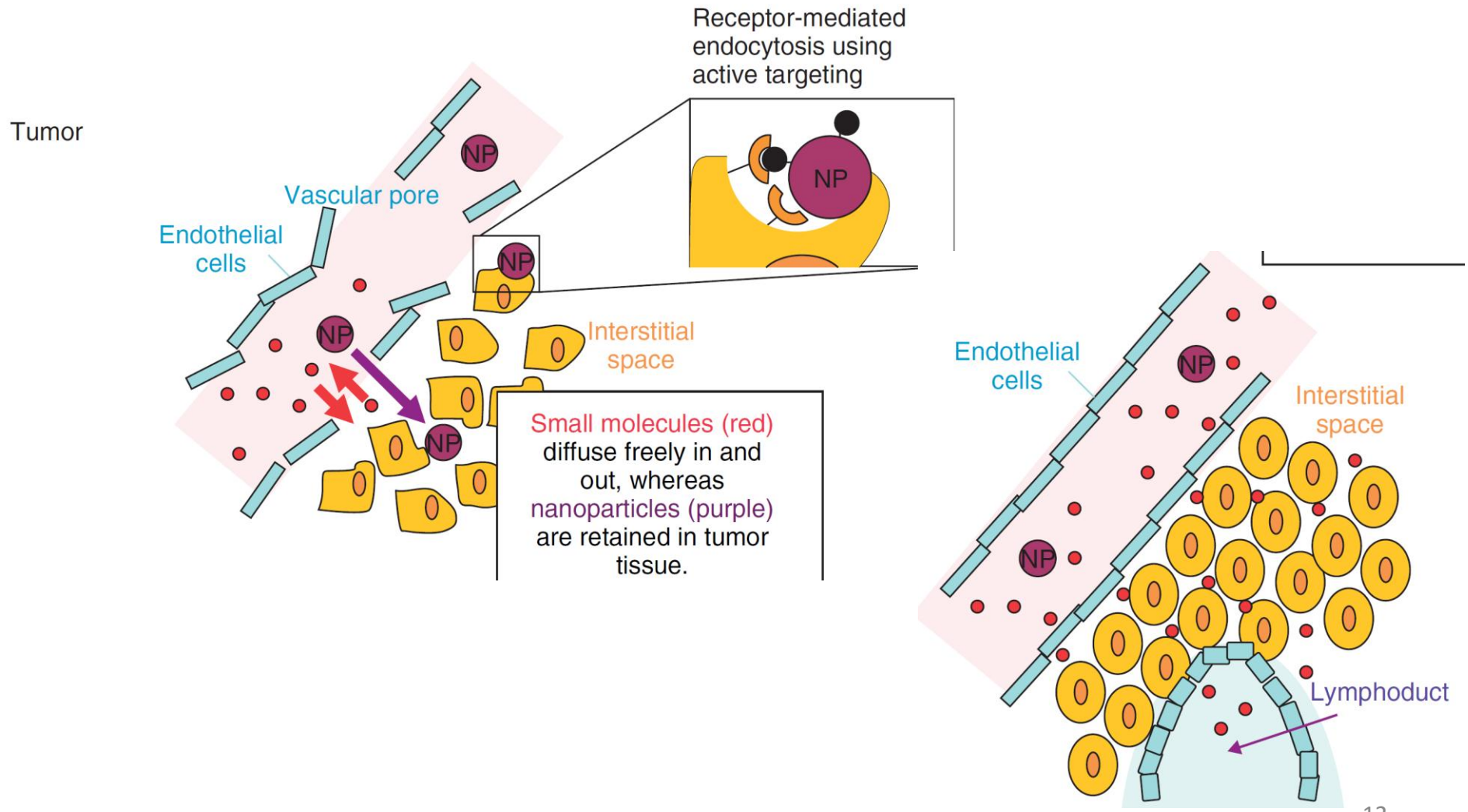
EPR effect (Passive targeting)

"Enhanced Permeation and Retention" effect

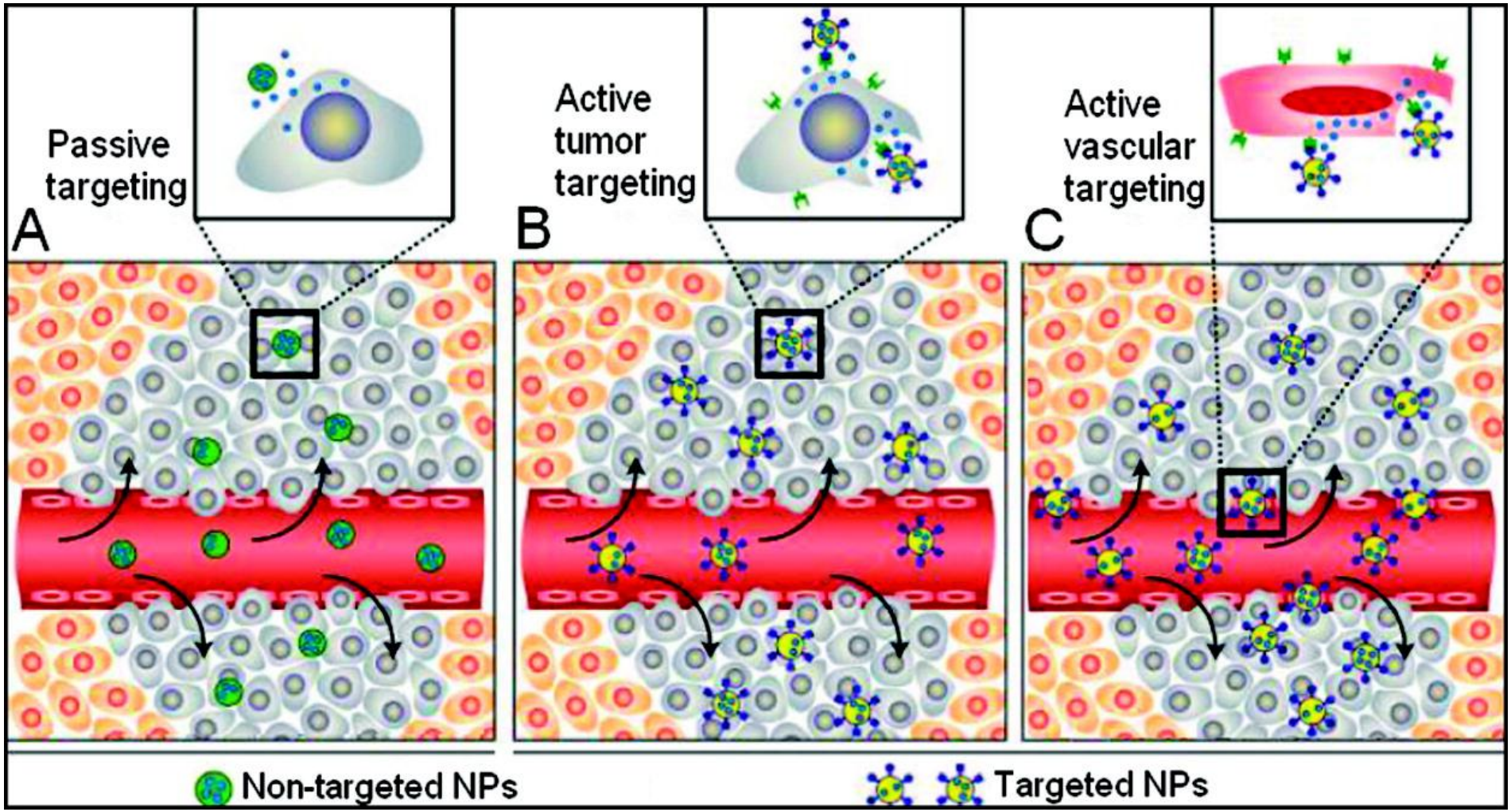


Pavan P. Adiseshaiah *et al. WIREs Nanomed Nanobiotechnol* 2009, 2, 99–112.

Limitations of Passive targeting

- Passively targeted NPs end up releasing their therapeutic payload into the tumor milieu rather than within cancer cells. (“PEG dilemma”)
- For drugs that are not readily retained in tumors or macromolecular drugs that are not readily taken up by cancer cells, this extracellular drug release may be less effective at maintaining a differentially high tumor drug concentration over an extended period of time.

Passive *vs* active targeting



Omid C. Farokhzad *et al. ACS Nano*, 2009, *3* (1), 16–20.

Active targeting

- Targeted NPs facilitate receptor-mediated endocytosis(RME), releasing therapeutic agents inside target cell.

- Higher therapeutic efficacy
- Lower toxicity

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NP Formulation Method

✓**"Bottom-up" (self-assembly)**

- Bulk synthesis
  - Nanoprecipitation
  - Oil-in-water emulsification-solvent evaporation
  - Water-in-oil-in-water emulsification-solvent evaporation, etc.
- Microfluidic synthesis

✓**"Top-down"**

- PRINT

  (Particle Replication In Non-wetting Templates)

Nanoprecipitation

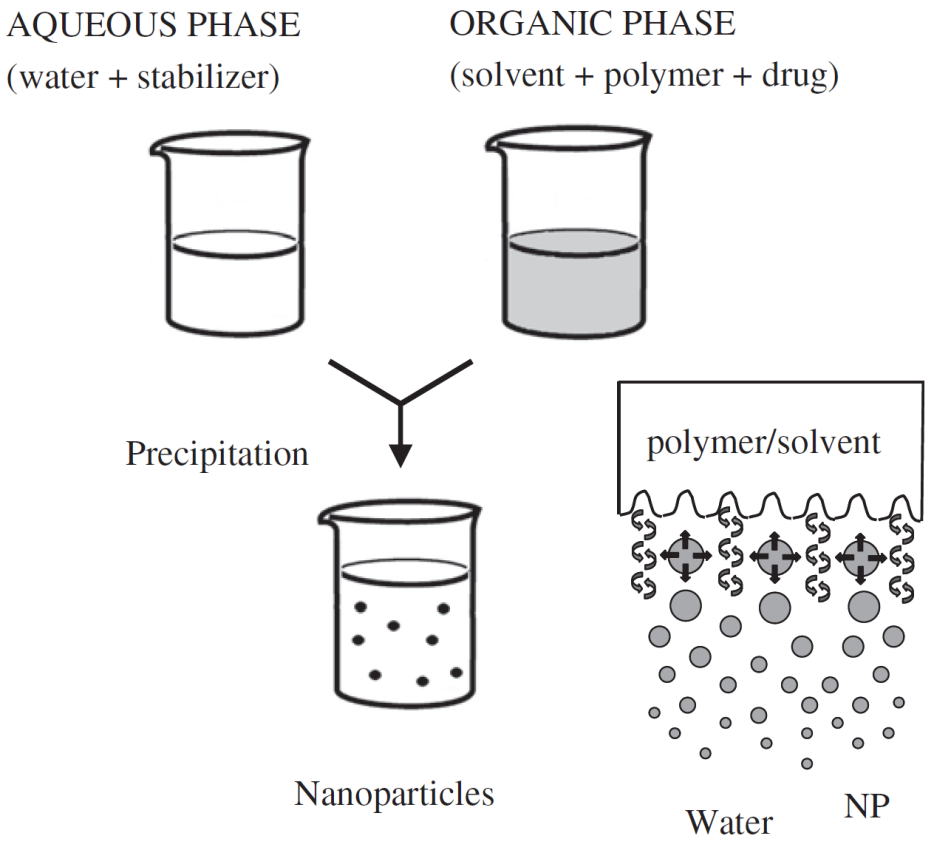
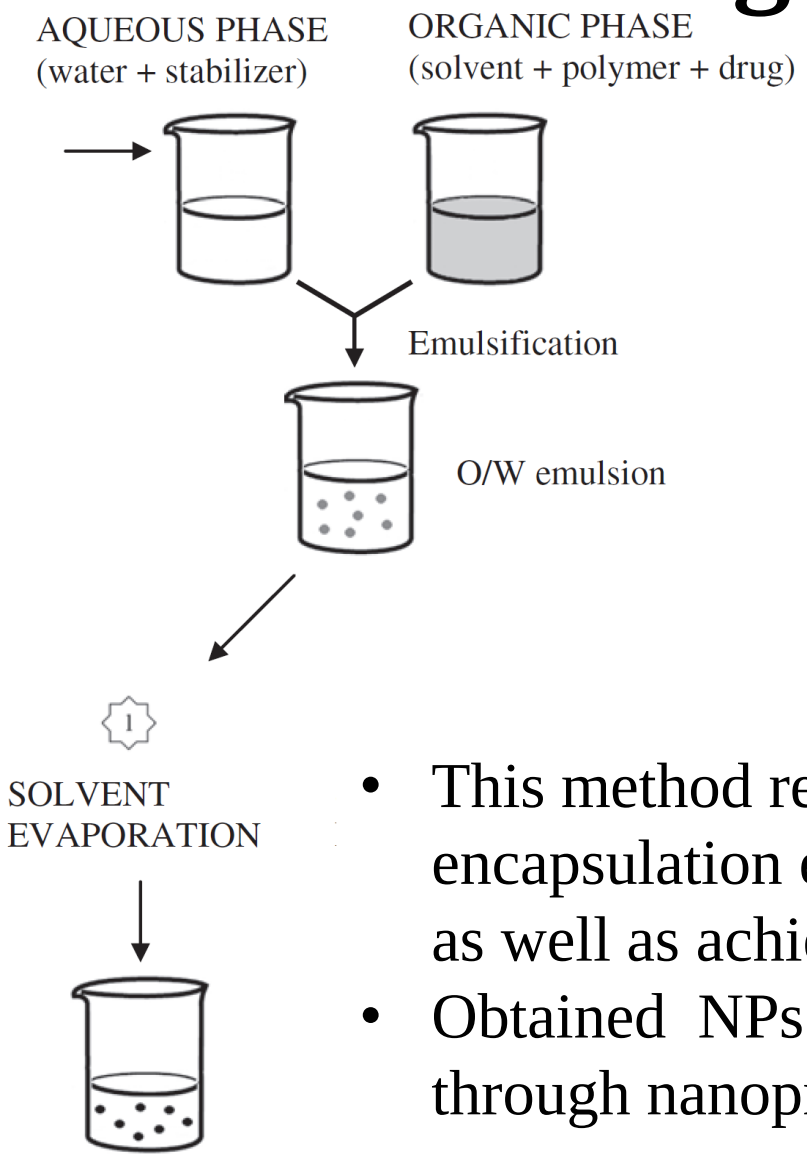


Figure 2. Nanoprecipitation or solvent displacement method.

- ✓ Difficulty in complete removal of the organic solvent after self-assembly.

Pegi Ahlin Grabnar *et al. Journal of Microencapsulation* 2011, *28*(4), 323–335.

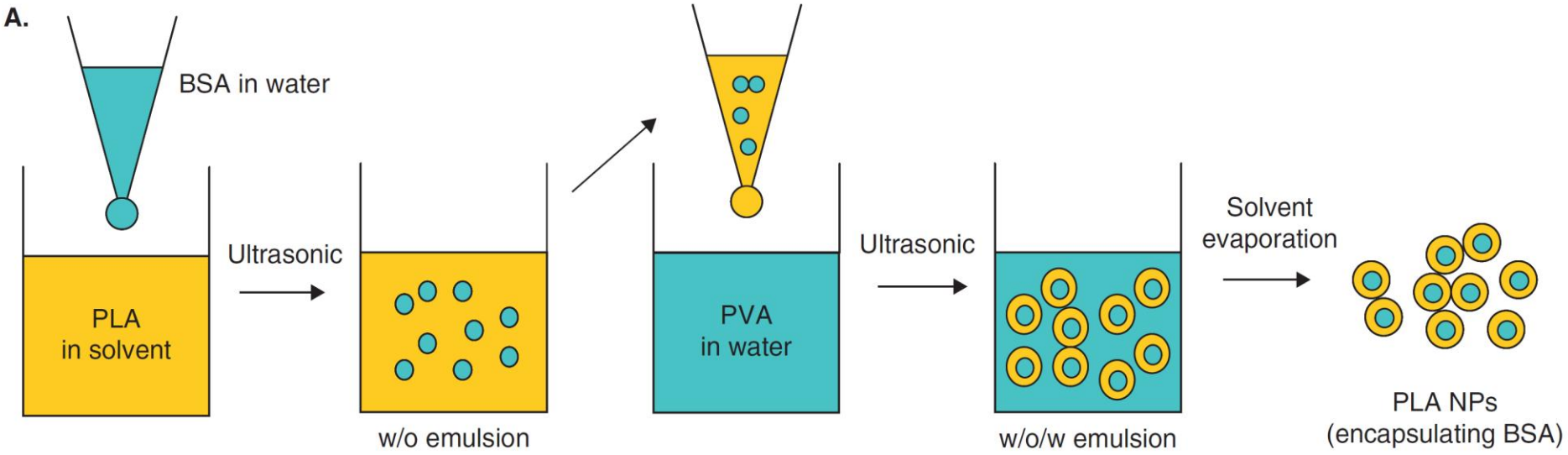
Single emulsion



- This method results in higher drug loading and encapsulation efficiency compared to nanoprecipitation, as well as achieving complete solvent removal.
- Obtained NPs are often larger than those obtained through nanoprecipitation.

Pegi Ahlin Grabnar *et al. Journal of Microencapsulation* 2011, *28*(4), 323–335.

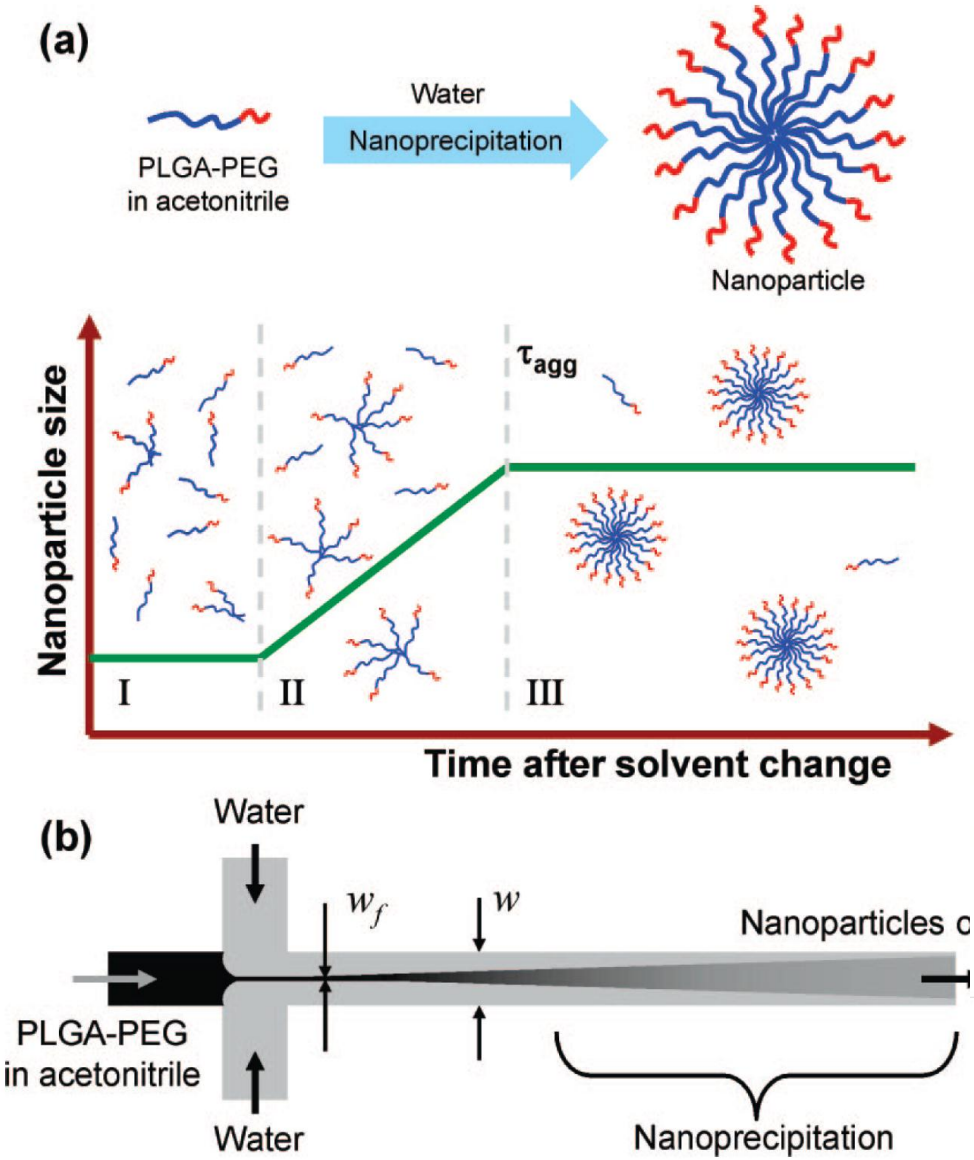
Double emulsion

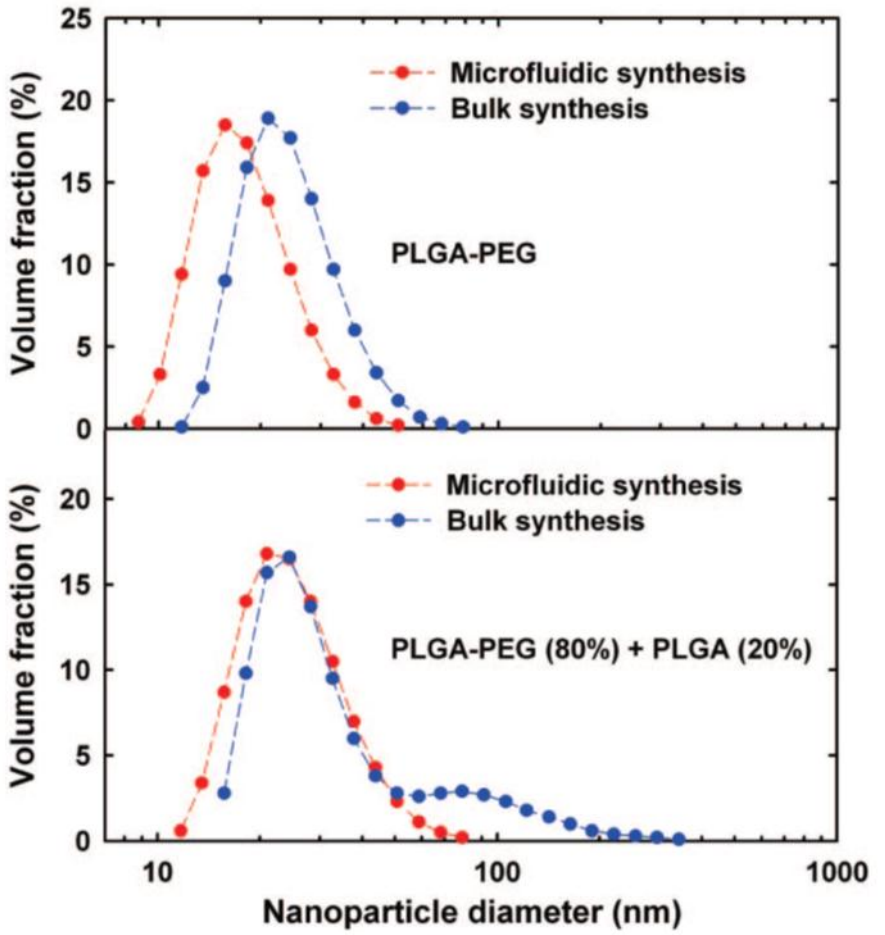


Guilin Wang *et al. Expert Opin. Drug Deliv.* 2008, *5*(5), 499-515.

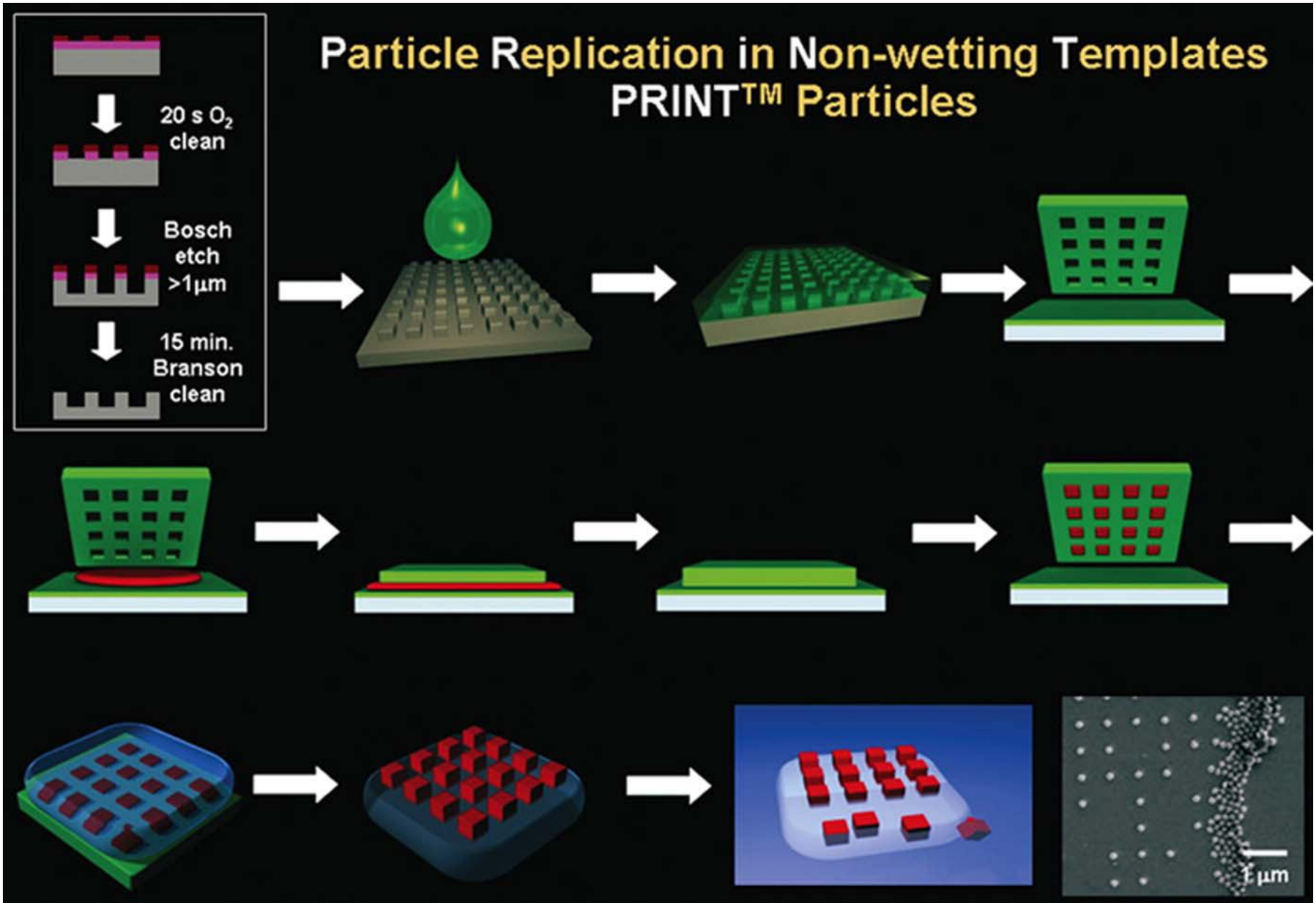
- This method is generally used for encapsulation of hydrophilic drugs
- This method normally yields NPs with larger size than nanoprecipitaion or O/W methods, with moderate drug loading and encapsulation efficiency.

Microfluidic methods

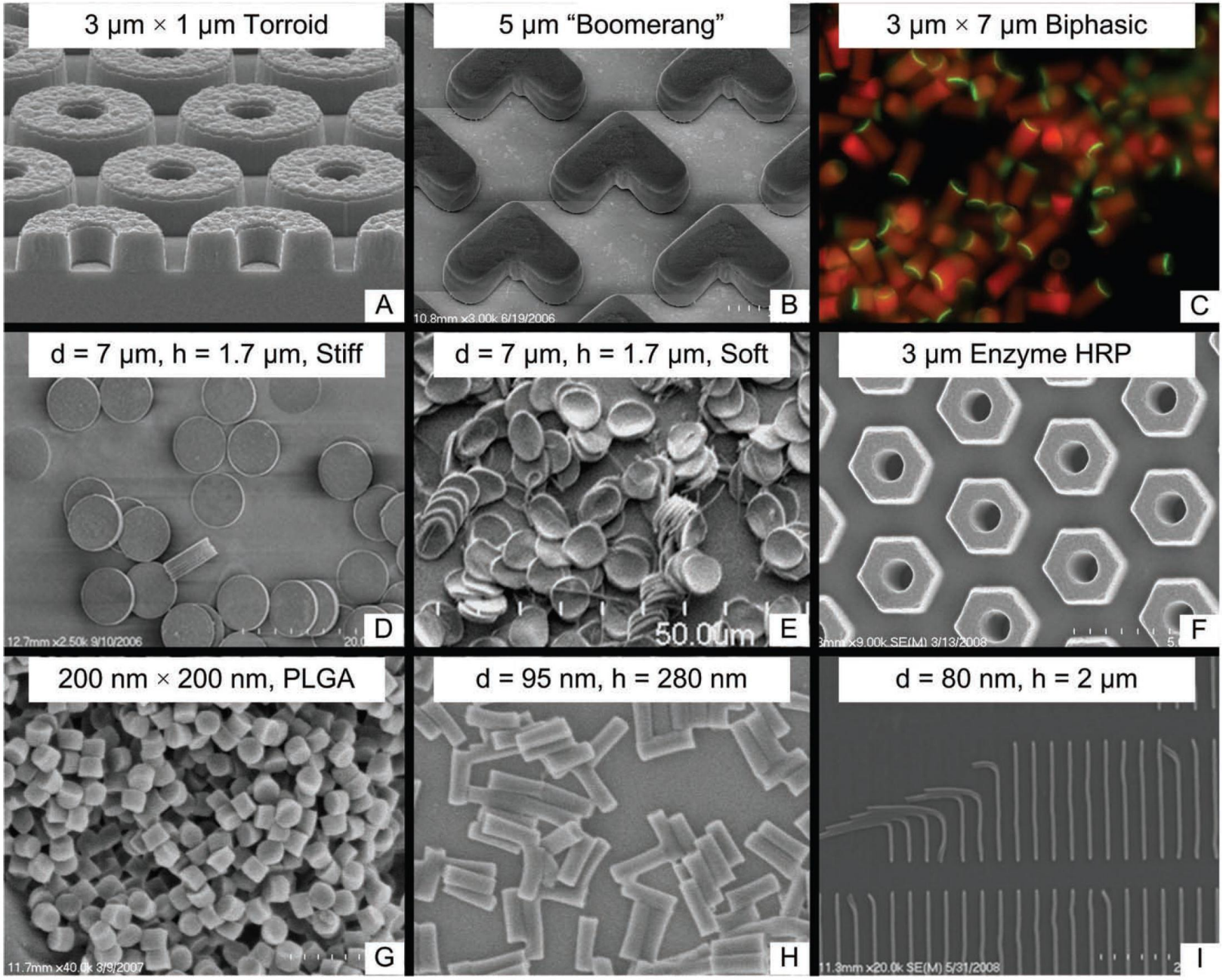




Rohit Karnik *et al*. *Nano Lett.* 2008, *8*(9), 2906-2912.

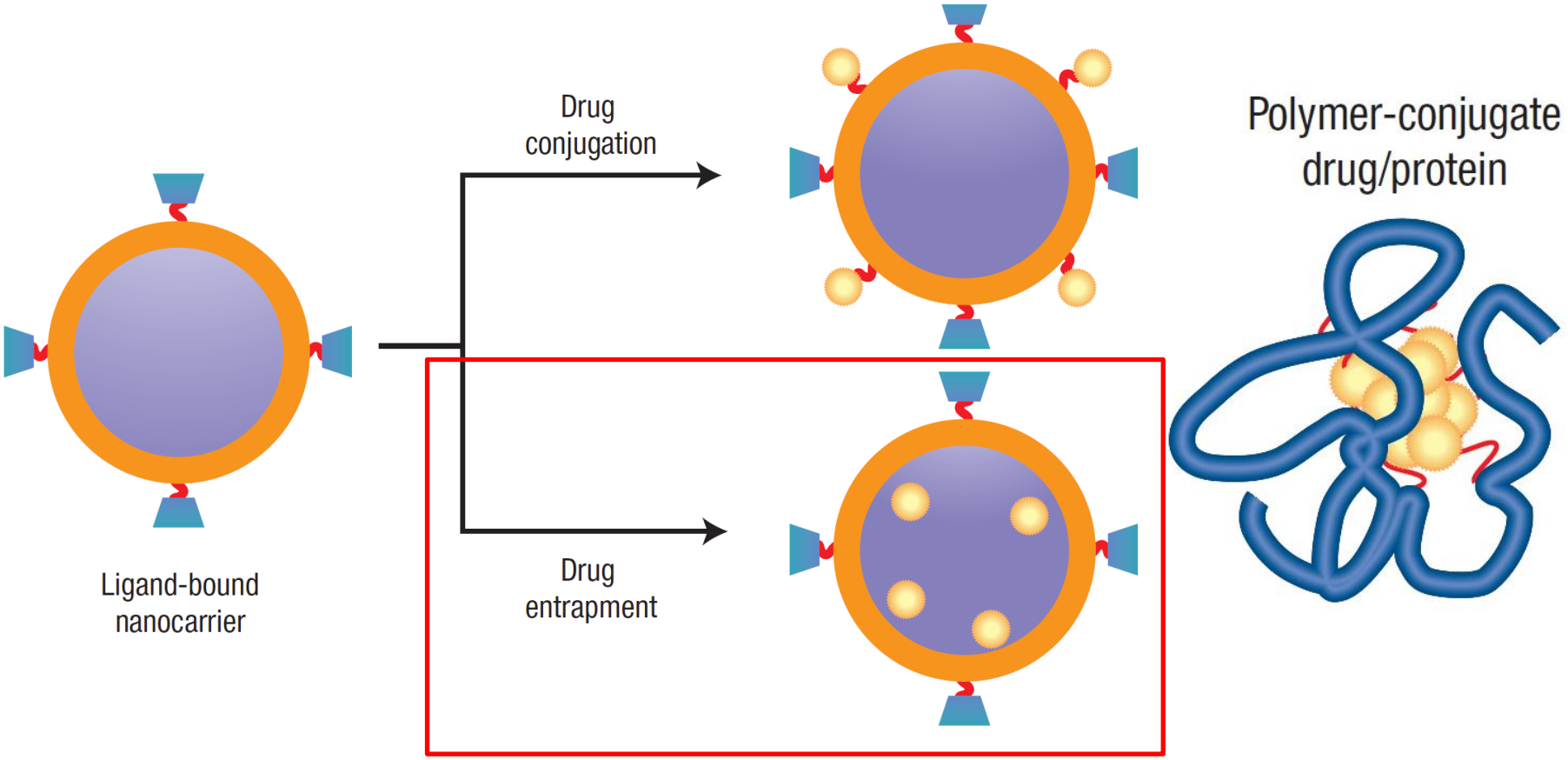


MARY E. NAPIER *et al. Polymer Reviews*, 2007, *47*, 321–327.



Jin Wang *et al. small*, 2011, *7,* No. 14, 1919–1931.

Drug loading methods

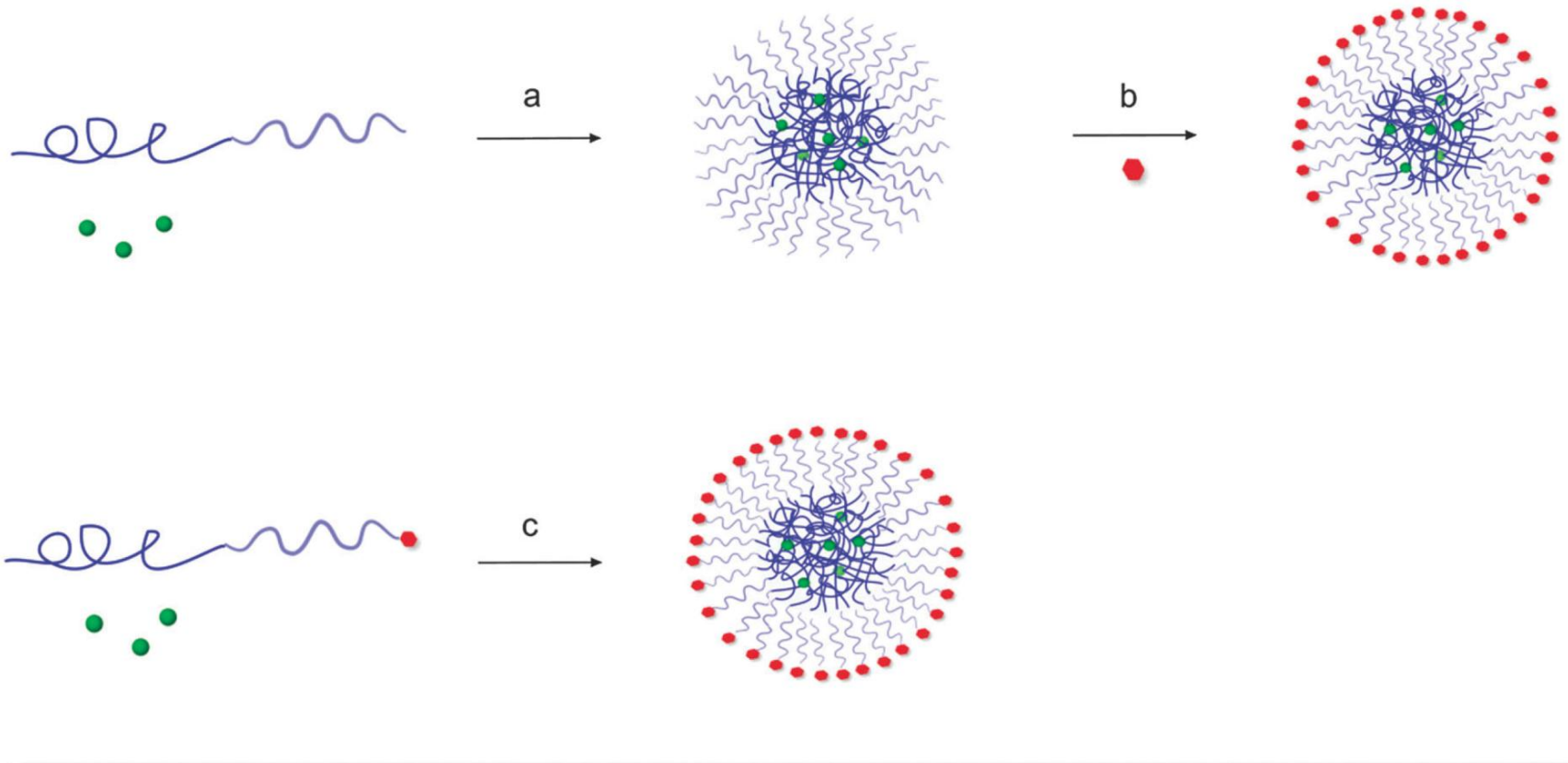


Encapsulation method is the most common technique in this field.

*The drug is entrapped in the polymer matrix during preparation of NPs.

Dan Peer *et al. nature nanotechnology*, 2007, *2*, 751-760.

Incorporation of targeting ligands on NPs



Nazila Kamaly *et al*. *Chem. Soc. Rev.*, 2012, *41*, 2971–3010.

Coupling chemistry should

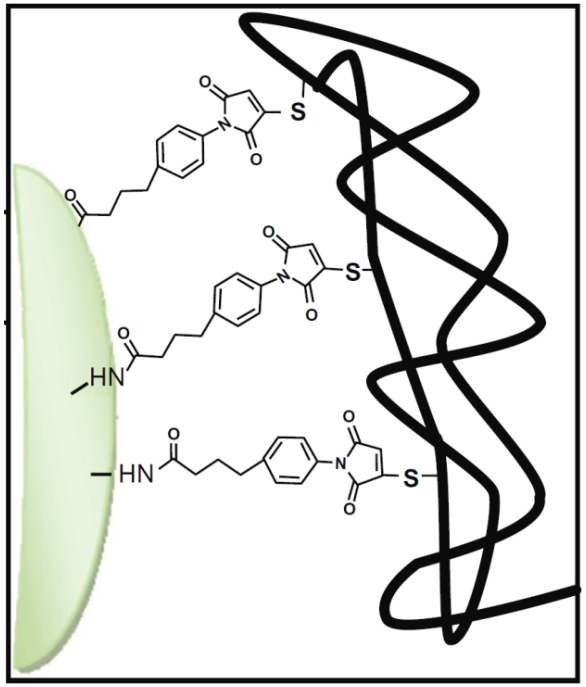
- not lead to undesirable products or side reactions
- be produced on large-scales in a reproducible manner

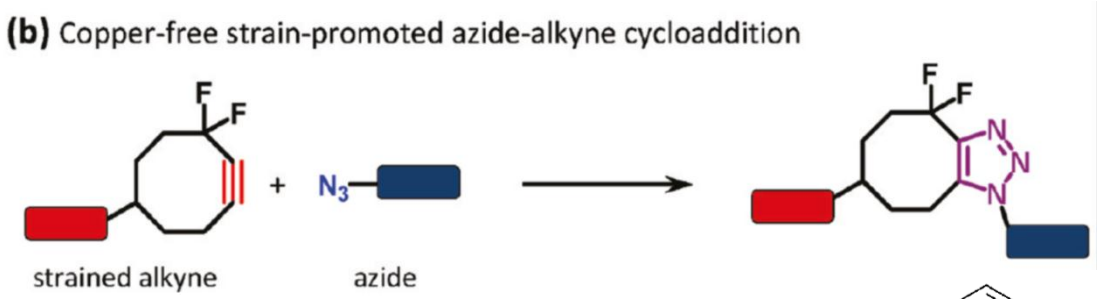
Post-synthesis NP surface modification method

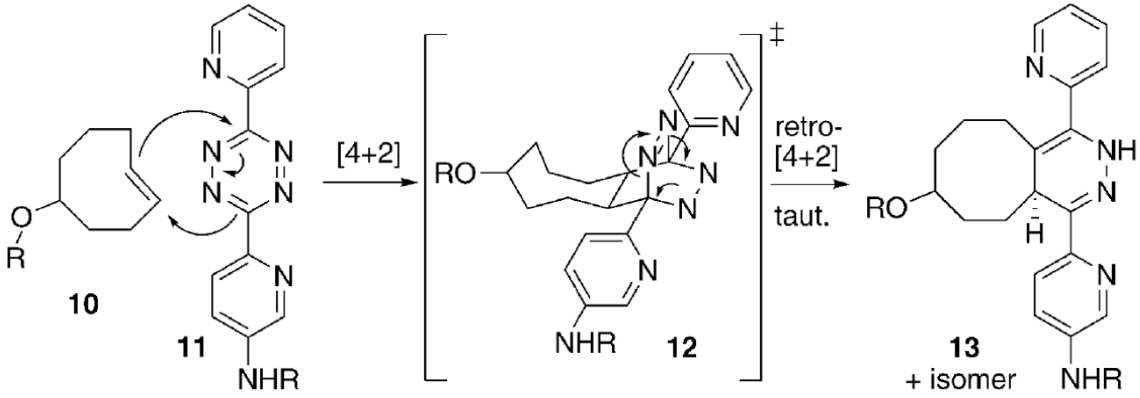
- Amide bond formation
- Maleimide coupling with thiols
- "Bioorthogonal" reactions such as

➢ Cu-free click reactions

➢ [4+2] cycloadditon reaction







W. Russ Algar *et al. Bioconjugate Chem*. 2011, *22*, 825–858.

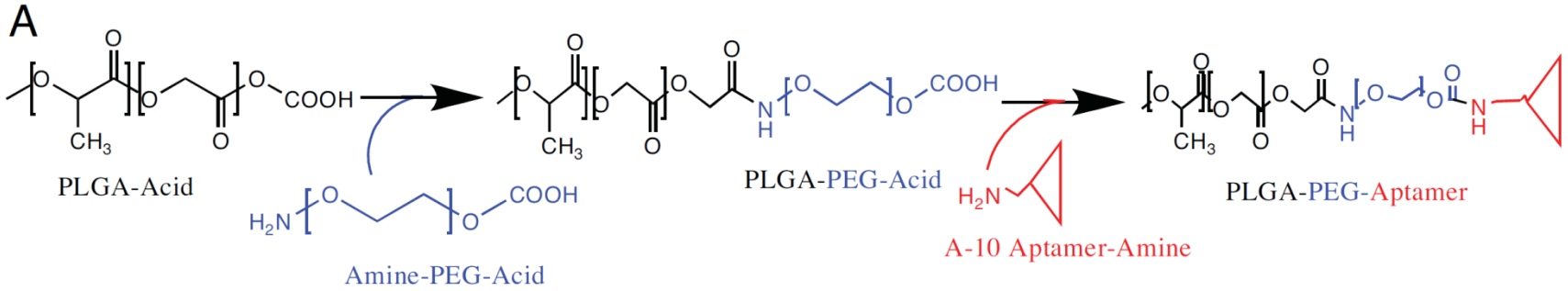
Mariagrazia Di Marco *et al. International Journal of Nanomedicine,* 2010, *5*, 37–49.

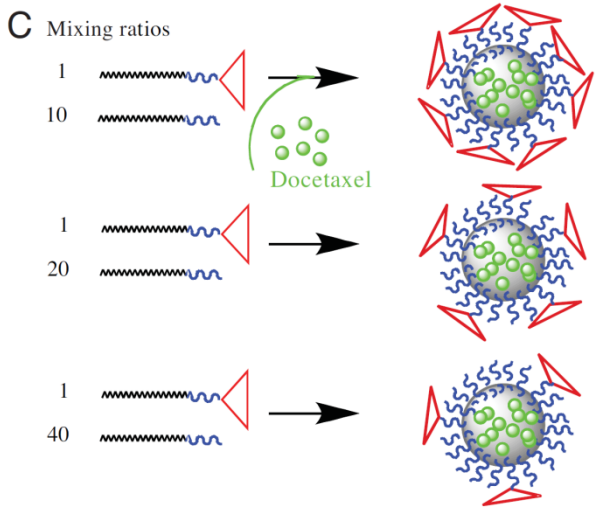
John C. Jewett *et al. Chem. Soc. Rev*., 2010, *39*, 1272–1279.

Targeted NPs through polymer self-assembly

- It is difficult to control the stoichiometry of functional biomolecules on the surface of NPs *via* coupling chemistries.

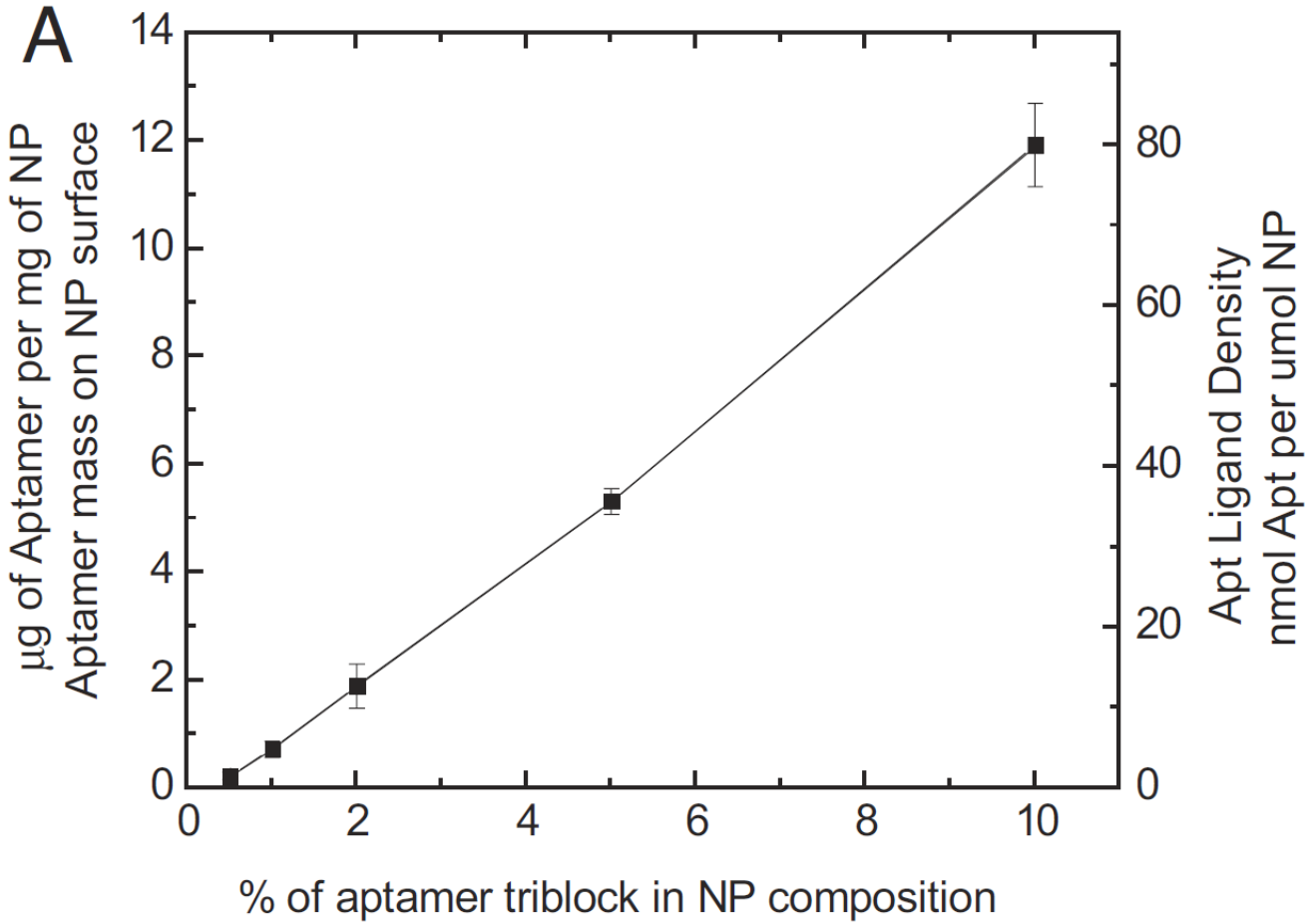
- **Self-assembly of pre-functionalized triblock copolymers** allows for the reproducible creation of optimal targeted NPs.





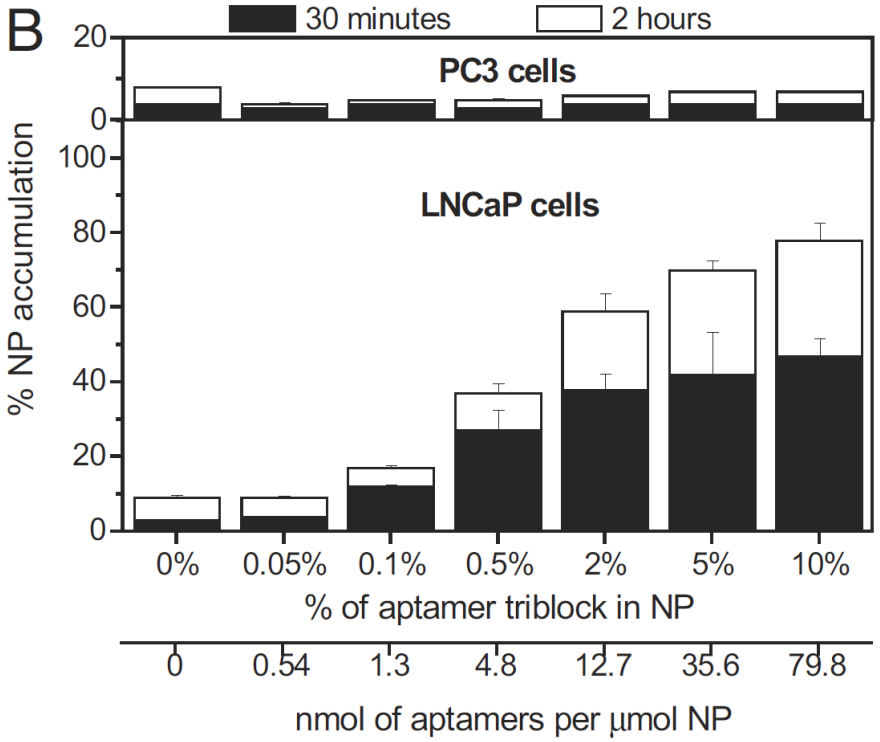
Frank Gu *et al. PNAS,* 2008, *105,* 2586-2591.

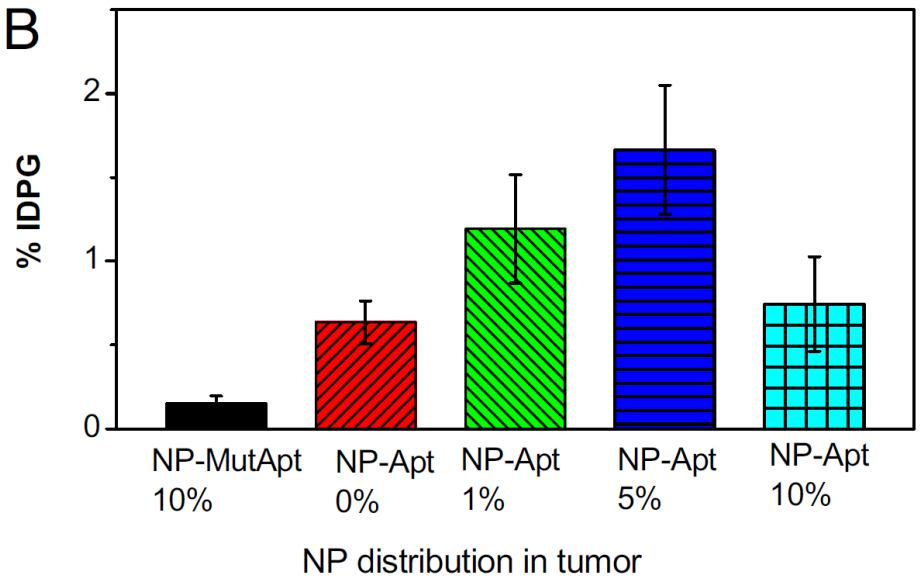
Precisely controlled aptamer density



Frank Gu *et al. PNAS,* 2008, *105,* 2586-2591.

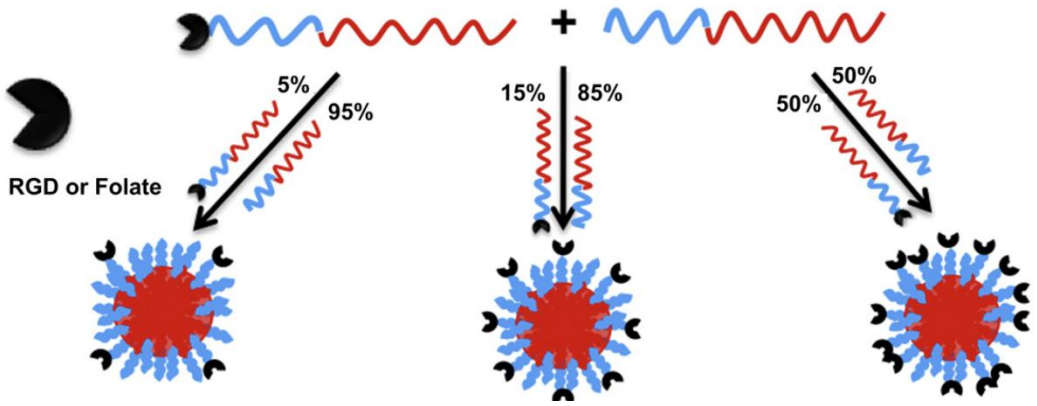
The effect of Apt surface density on NP *in vivo vs in vitro*

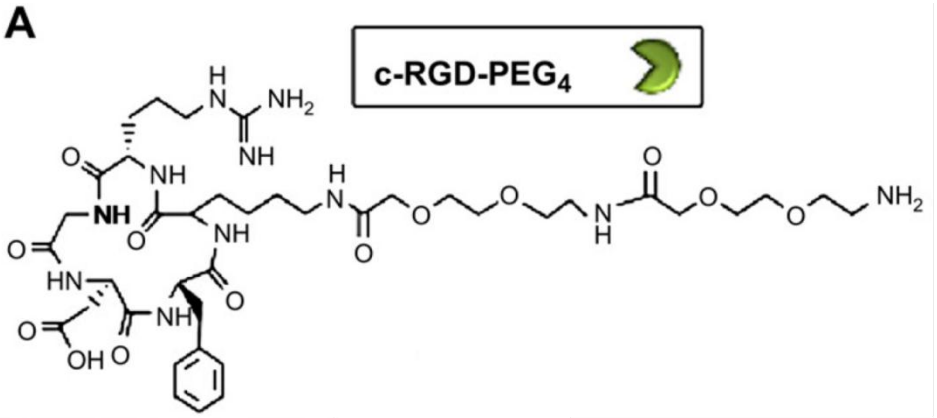


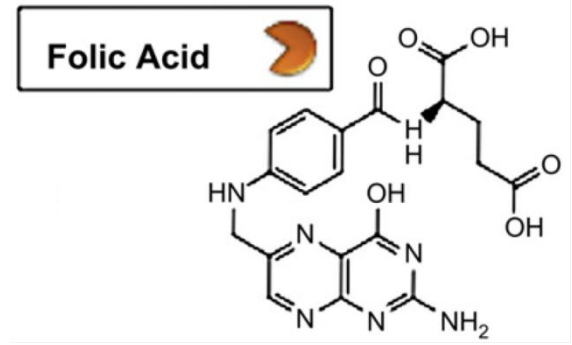


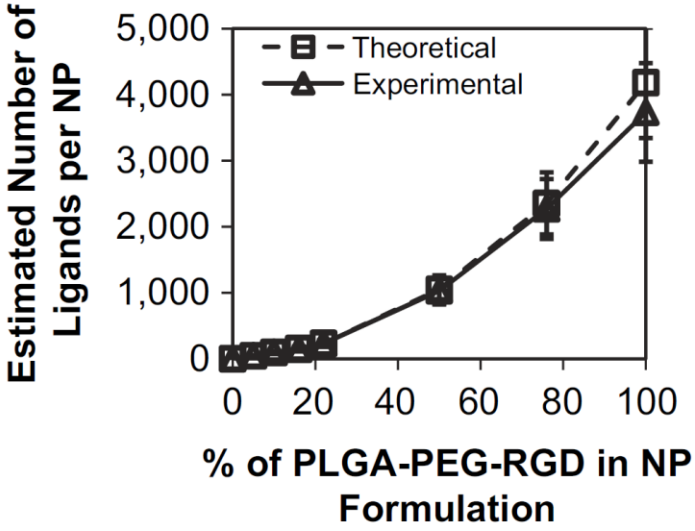
Frank Gu *et al. PNAS,* 2008, *105,* 2586-2591.

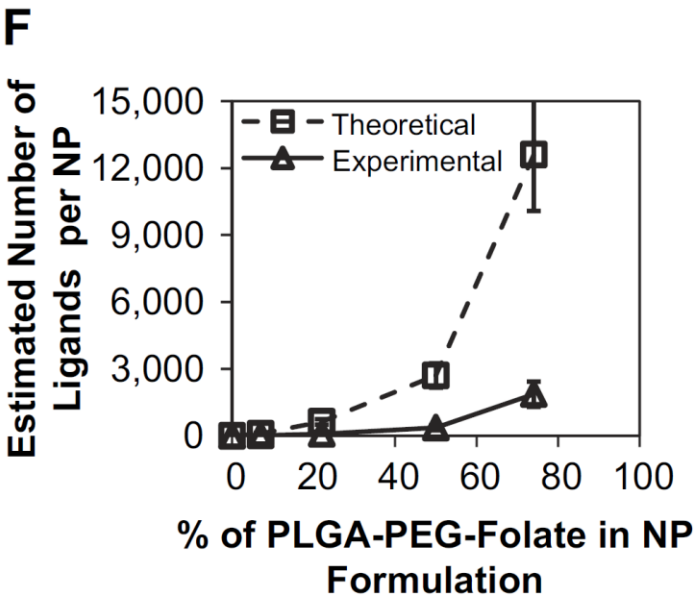
Solubility of ligands











Pedro M. Valencia *et al. Biomaterials*, 2011, *32*, 6226-6233.

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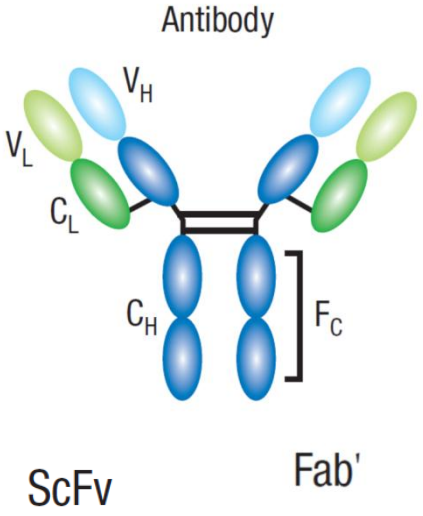
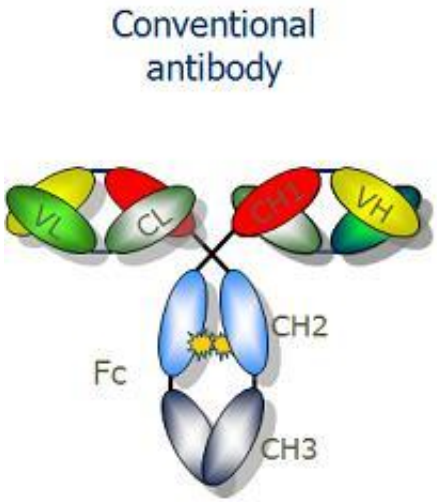
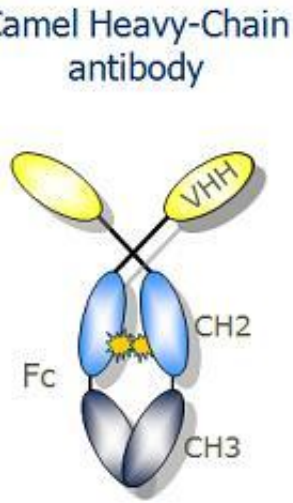
Targeting ligands

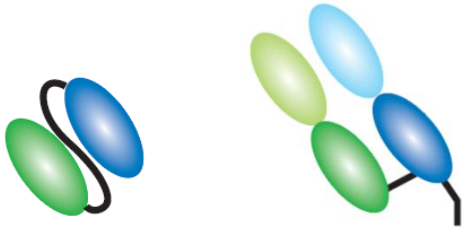
- Antibodies and their fragments
- Proteins
- Peptides
- Aptamers (Nucleic acid lidands)
- Small molecules (folic acid, carbohydrate etc.)

Antibodies and their fragments









Dan Peer *et al. nature nanotechnology,* 2007, *2,* 751-760.

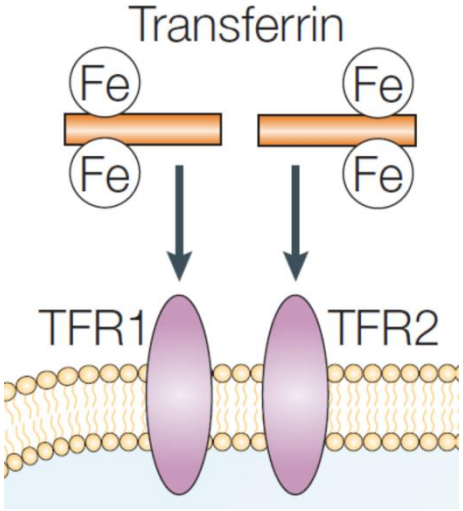
Type	MW/kDa	Diameter/nm
Whole antibodies	150	15-20
Fab’	50	5-10
ScFv	25	3-5
Nanobody	15	2-3

Proteins

- Endogenous proteins that selectively bind to specific membrane-bound receptors on cells can be used.

➢ Transferrin, Epidermal Growth Factor, Nerve Growth Factor, etc.

☐ The receptors of Tf and EGF are overexpressed on cancer cells.



✓ Demerits

Commonly immunogenic, off-target adverse effects

Ulrich E. Schaible *et al.* NATURE REVIEWS, 2004, *2*, 946-953.

Peptides

- Small size, relatively low immunogenicity, high stability, and ease of conjugation to NP surfaces

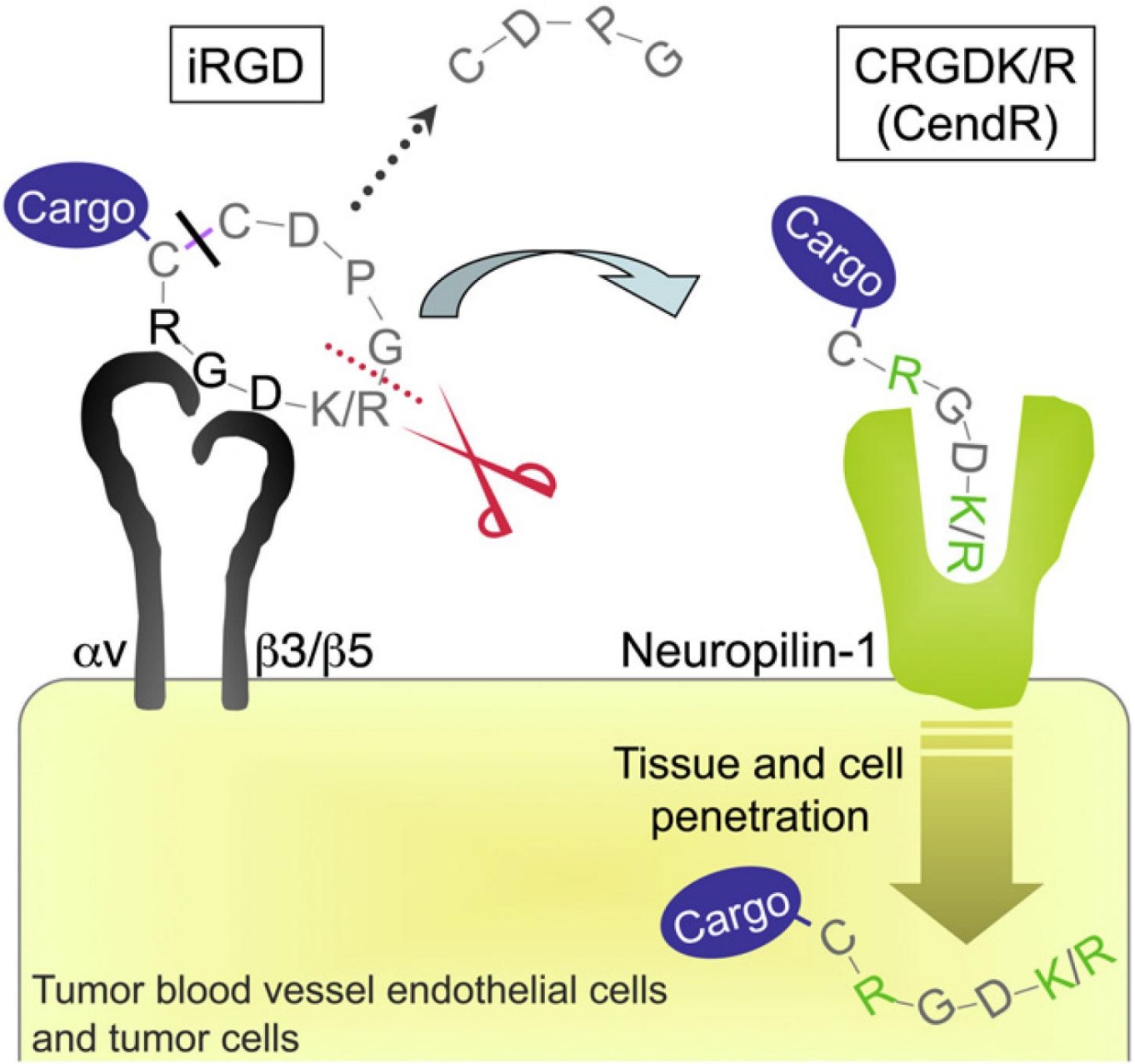
➢ RGD (Arg-Gly-Asp) sequence binds to $\alpha_v\beta_3$ integrin receptors which are highly upregurated on both tumor cells and angiogenic endothelial cells.

➢ Cell-penetrating peptides such as Tat peptide

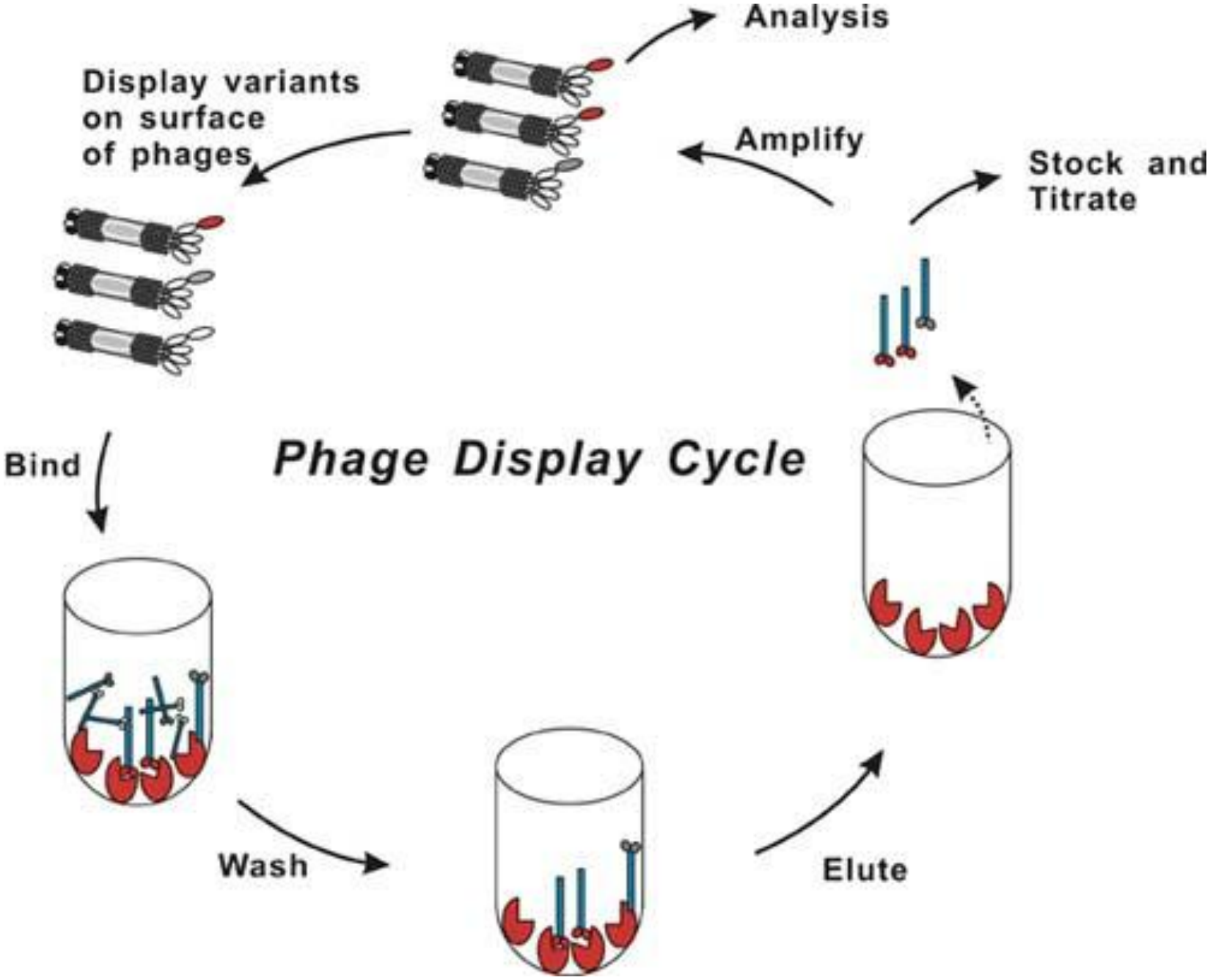
☐ Tat peptide derives from the HIV-1 virus.

➢ Peptides with R/KXXR/K motif such as iRGD

☐ iRGD homes to tumors and penetrates into them.



Kazuki N. Sugahara *et al. Cancer Cell*, 2009, 16, 510–520.



http://www.creative-biolabs.com/phagedisplay1.htm

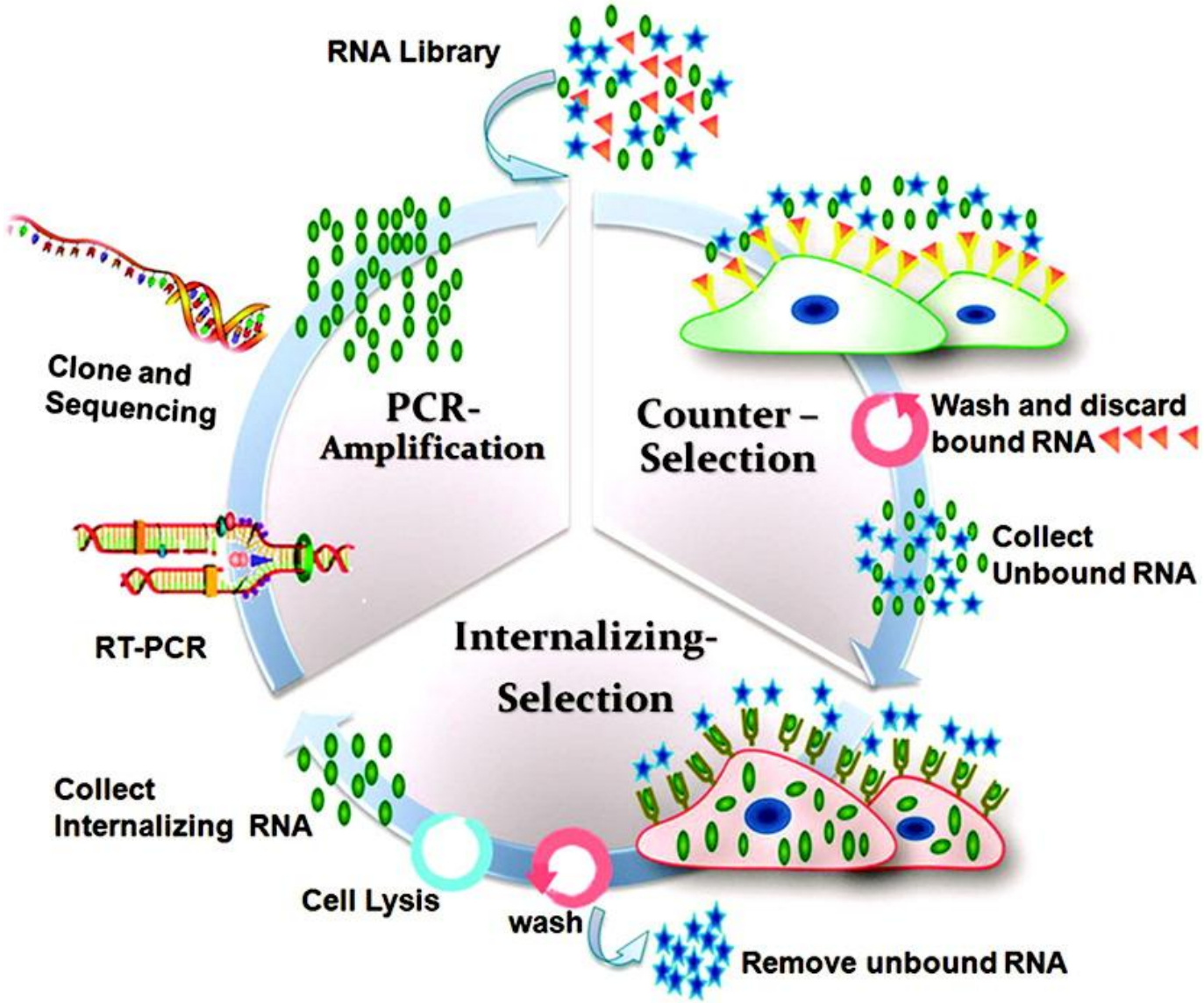
Aptamers

- Single-strand of DNA or RNA oligonucleotides
- Small size, reproducible synthesis, low immunity

Type	MW/kDa	Diameter/nm
Whole antibodies	150	15-20
Nanobody	15	2-3
Aptamers DNA/RNA	10-30	2-3

✓ The high specificity of Apts against targets is their secondary structure, but the secondary structure may be affected by heat, exonuclease or endonuclease degradation.

“cell-uptake selection”



Z. Xiao *et al*. *ACS Nano*, 2012, *6*, 696–704.

Small molecules

- The availability of a range of facile coupling chemistries for their conjugation
- The availability of a wide range of targeting ligands with variable solubilities and functional groups

➢ Folic acid (or folate)

Folate receptors (FRs) are frequently over-expressed in a range of cancer

✓ FRs are expressed not only in tumor tissue but in normal epithelia.

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Influence of particle size

- The generally accepted diameter of nanomedicine for cancer is in the range of 10-100 nm.

✓ The lower limit is determined by an interaction with renal filtration in the kidney.

✓ The upper limit is determined by an interaction with RES (immune system) in the spleen and liver.
(particles larger than 200 nm must compensate by deformability)

✓ For the purpose of tumor accumulation, the upper limit for extravasation into solid tumors have been suggested at ~400 nm.

- **Influence of NP shape**

✓ Spheres *vs* Rods on cellular uptake?

➢ Further investigations are required.

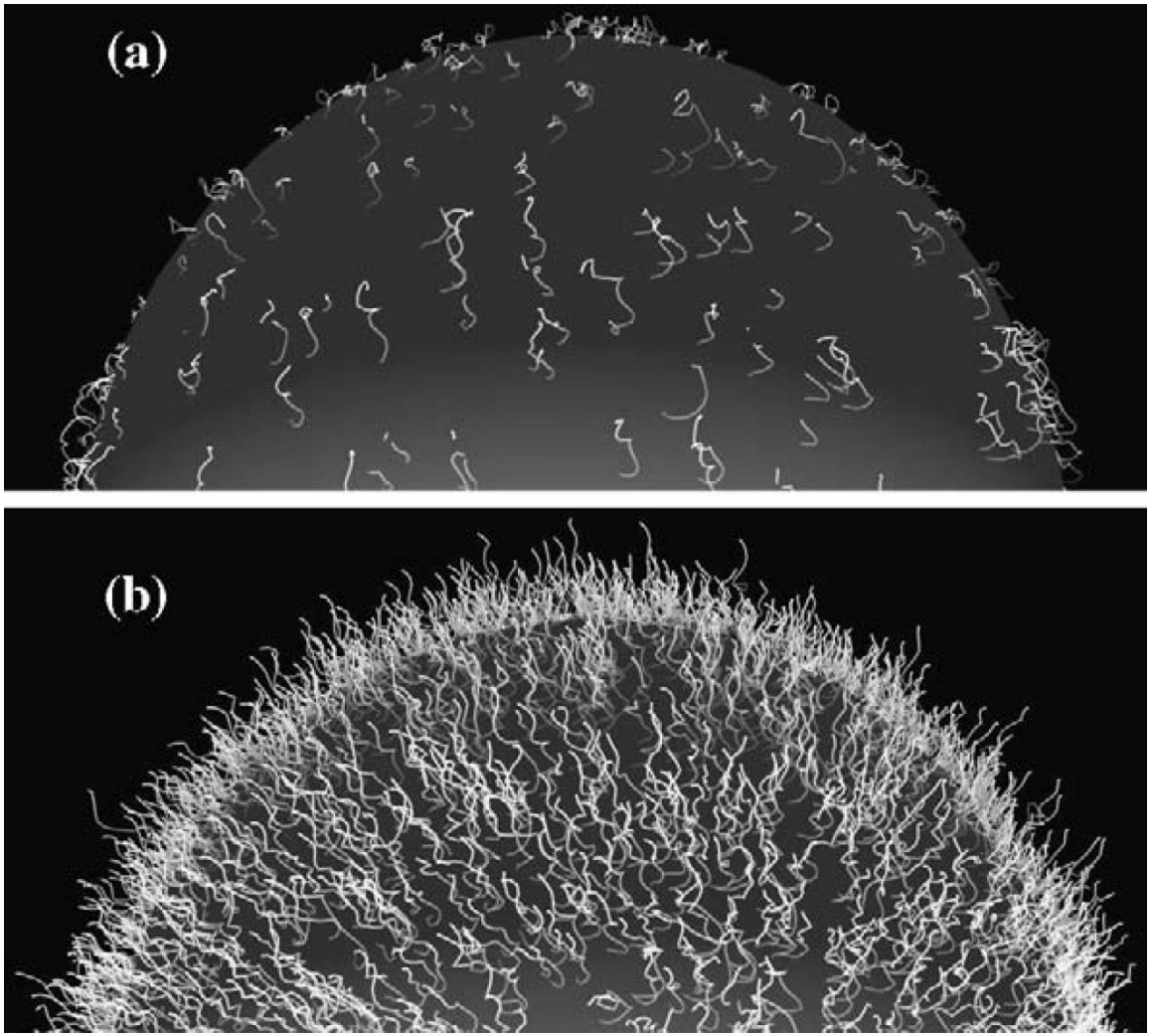
- **Influence of NP surface charge**

✓ NP surface charge is a major factor contributing to the non-specific binding of NPs to cells.

✓ Charged NPs will inevitably have short half-lives and high non-specific cellular uptakes due to interaction with blood proteins and complement activation.

➢ Neutral particles would be good.

Influence of NP PEGylation



“mushroom”

Optimal PEG coverage?

“brush”

Donald E. Owens III *et al. International Journal of Pharmaceutics*, 2006, *307*, 93–102.

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“A new class of therapeutics”

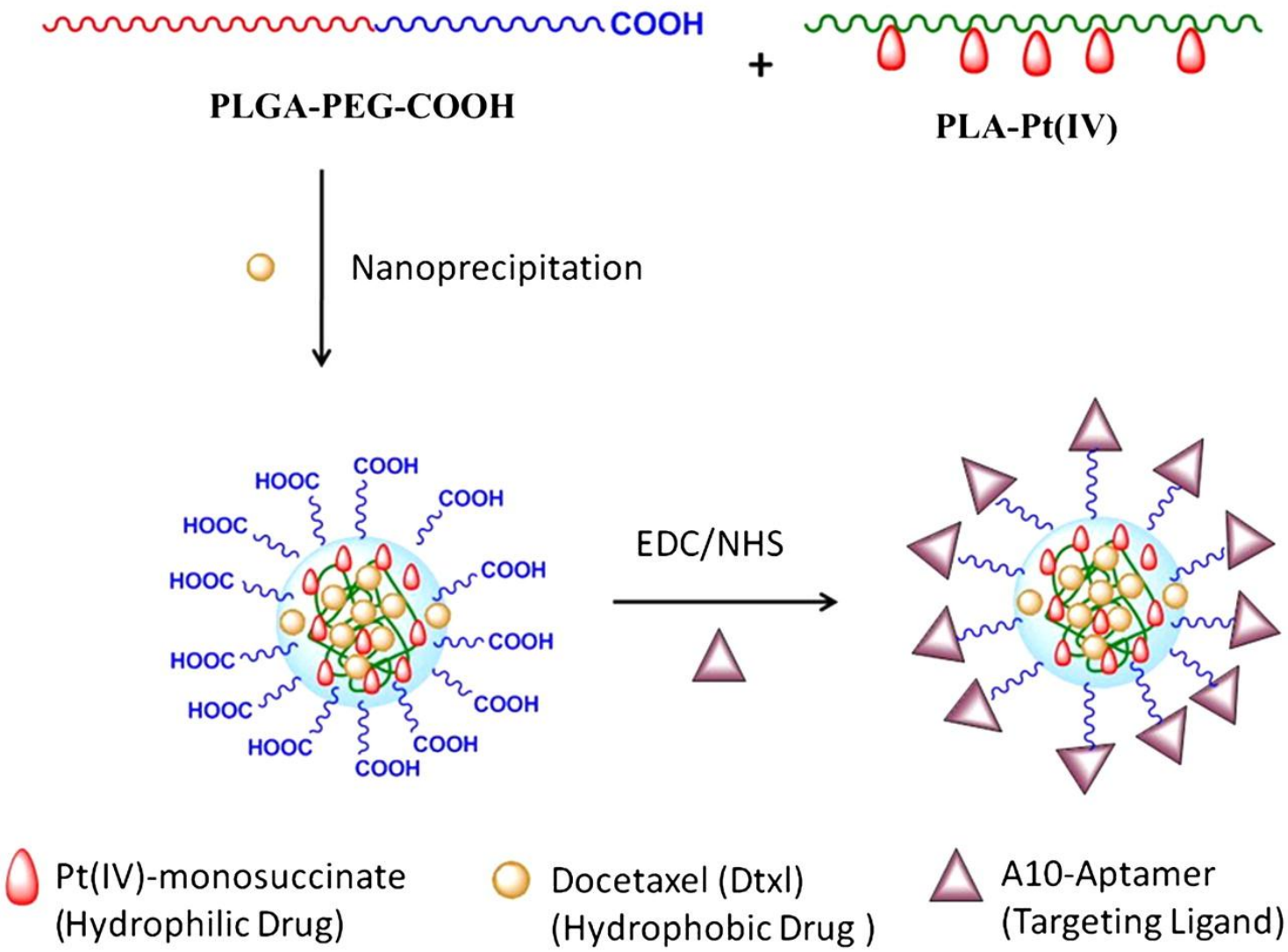
- Delivering therapeutics in a more controlled and specific manner

➢ Improved drug safety and efficacy

- Protecting drugs from rapid metabolism and inactivation; improving drug solubility, PK, BD, and target tissue exposure

➢ Additional degrees of freedom to medical chemistry

Co-delivering of multiple drugs



N. Kolishetti *et al.* Proc. *Natl. Acad. Sci. U. S. A*., 2010, *107*, 17939–17944.

Challenges

- ✓ Insufficient understanding of events at the nano-bio interface *in vitro* and *in vivo*
- ✓ Inadequate knowledge of the fate of NPs at the body, organ, and cellular levels
- ✓ Difficulty in achieving reproducible and controlled synthesis of NPs at scales suitable for clinical development and commercialization
- ✓ Overreliance on the EPR effect (This phenomenon may not be a universal property of all tumors.)
- ✓ There are too many “on a case-by-case basis”.
- ➢ Is it possible for a reasonable strategy to exist?

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