PAPER PRESENTATION

BY

KAMALESH

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Gold, Poly(β-amino ester) Nanoparticles for Small Interfering RNA Delivery

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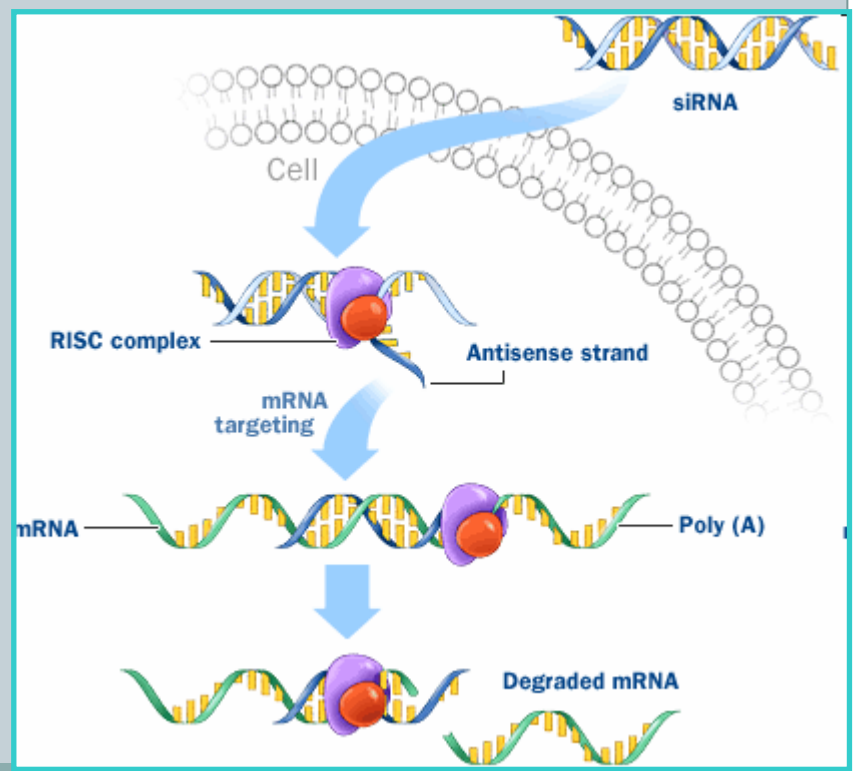
RNA interference -

- RNA interference is a system within living cells that helps to control which genes are active and how active they are.
- It is an endogenous process whereby double-stranded RNA (dsRNA) can mediate the catalytic destruction of its homologous mRNA target.
- RISC – RNA induced silencing complex



Need for work-

- The safe and effective intracellular delivery of siRNA remains the most challenging barrier to the broad application of siRNA in the clinic.
- To prove use of PBAEs as a delivery enhancer, and gold nanoparticles (AuNPs) as a scaffold to assemble siRNA strands.

Plan -

- Step 1 : Preparation of NH2-PEG-AuNPs
- Step 2 : Conjugation of HS-siRNA to NH2-PEG-AuNPs by biodegradable disulfide linkage
- Step 3 : Quantitative analysis of #(siRNA)/AuNP
- Step 4 : Optimization of reaction conditions
- Step 5 : Preparation of PBAE
- Step 6 : Preparation of Au-siRNA-PBAE nanoparticles:
- Step 7 : Cellular transfection and gene knockdown

Preparation of NH2-PEG-AuNPs

- The synthesis of the siRNA-AuNP begins with modifying AuNPs with HS-(CH2CH2O)n-NH2 , (M.W.~1000Da)

AuNP (colloid 15nm, ~2.5nM) + HS-PEG-NH2 (20mg) → 12hr incubation At 25° C

(washing and redispersion in PBS containing 0.01 % polysorbate surfactant Tween20 for further use)

Tween 20 is a polysorbate surfactant whose stability and relative non-toxicity allows it to be used as a detergent and emulsifier in scientific, and pharmacological applications.

Conjugation of HS-siRNA to NH2-PEG-AuNPs

- Conjugation of HS-siRNA to NH2-PEG-AuNPs
  - conjugation of SPDP to NH2-PEG-AuNPs
  - conjugation of HS-siRNA to SPDP-PEG-AuNPs

i) SPDP - *N*-succinimidyl 3-(2-pyridyldithio) propionate

NH2-PEG-AuNPs + SPDP → 25°C

(400μL, 30 nM) (400 μL, 3 mM in PBS, 10 %DMSO) → 40min. vigorous vortexing

(washing with PBS containing 0.01 % Tween20 to get pallete)

Conjugation of HS-siRNA to NH2-PEG-AuNPs

ii) siRNA - antifirefly luciferase

A = HS-siRNA + NaCl + Borate buffer + Tween 20

(15 µM) (2.5 M) (400 µL,pH 8.5,30 mM) (0.01 %)

B = SPDP-PEG-AuNPs pallete

25°C, 40 hrs

A + B ⟶ siRNA-AuNPs

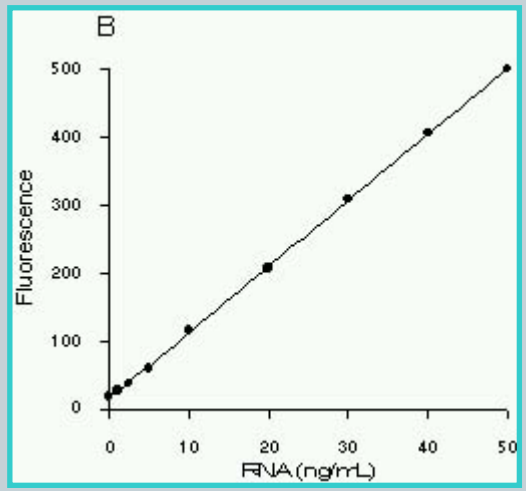
(400µL) (pallete) vigorous vortexing

washing with PBS 0.01 % Tween 20

Concentration was adjusted to 15nM

Quantitative analysis of #(siRNA)/AuNP :

- siRNA-AuNPs were incubated in 0.05 M dithiothreitol solution in PBS (pH 7.4, 0.01 % Tween 20) for 30 min at 30 ^{o}C to cleave the disulfide bonds.
- Number of released siRNA strands were quantitatively analyzed by RiboGreen™ RNA reagent.

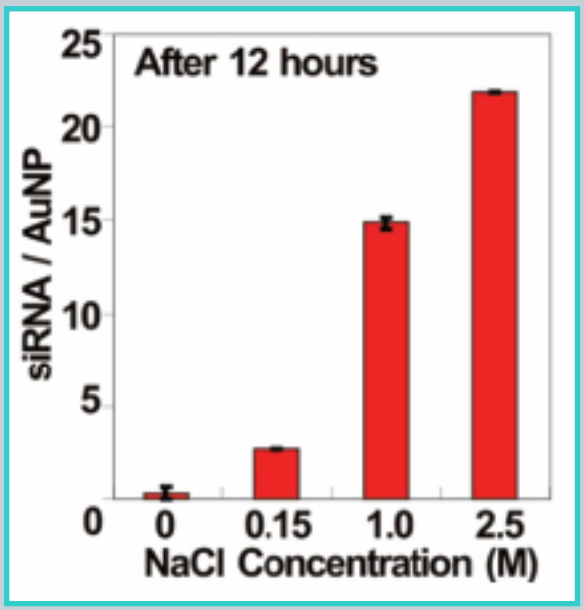


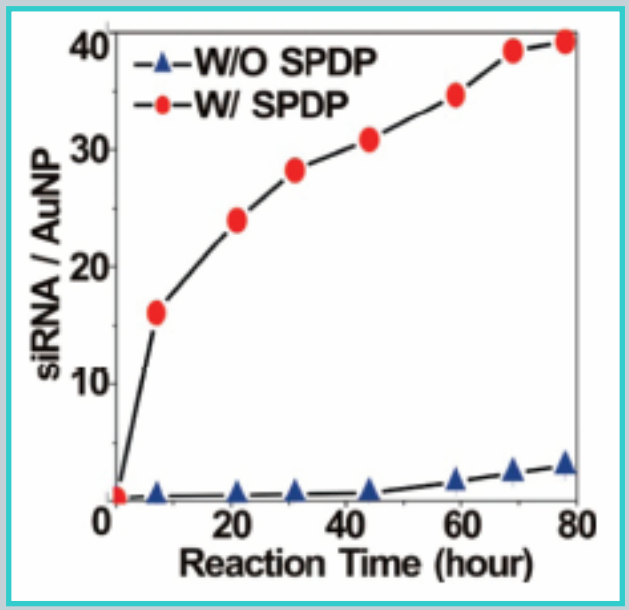
- Protocol is available at, http://www.thelabrat.com/protocols/DNA-RNAQuantitation4.shtml

Optimization of reaction conditions

- Optimization of reaction conditions to increase loading of siRNA on AuNP,

  High salt stability(up to 2.5M NaCl) of NH2-PEG-AuNPs was important to facilitate siRNA conjugation

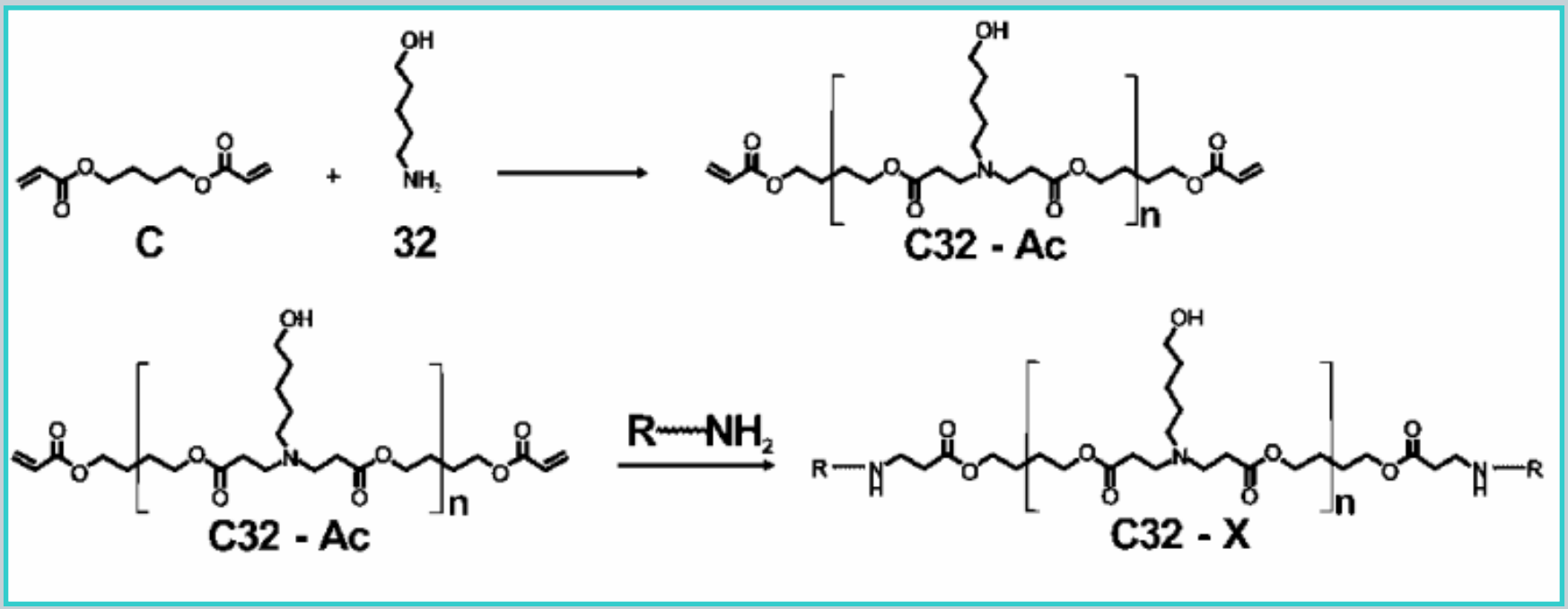




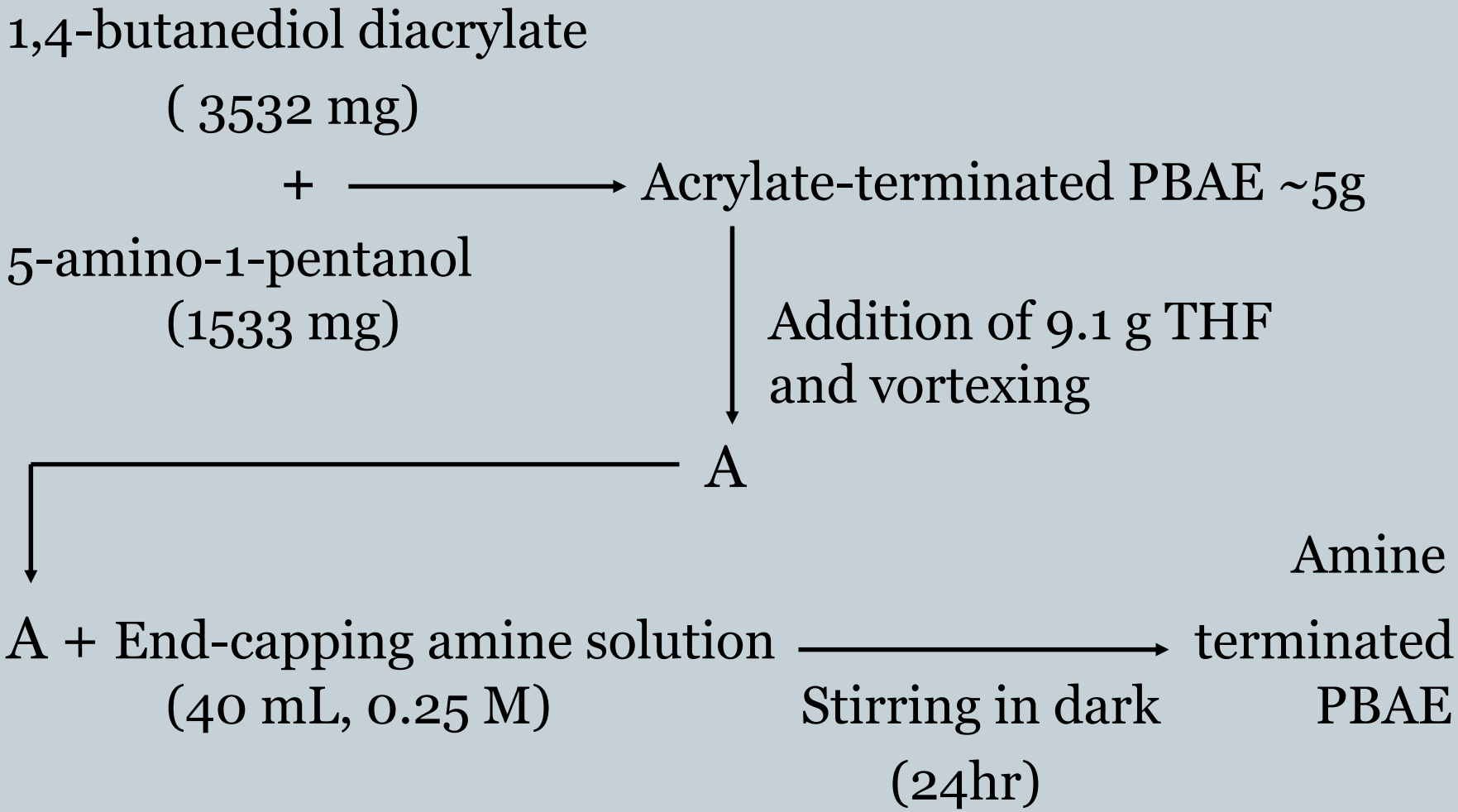
Optimization of reaction conditions

- Increase in salt concentration helps to reduce repulsion between the negatively charged siRNA strands and hence increases loading.
- Loading of siRNA per AuNP without SPDP was low, indicates that HS-siRNA is conjugated primarily to SPDP, not to the AuNP surface by displacing NH2-PEG-SH from gold.
- Slight formation of AuNP aggregates takes place after 40 hrs, hence the optimum conjugation time for HS-siRNA to AuNPs was determined to be 40 hrs
- Optimization results into ~30 strands of siRNA per particle.

Preparation of PBAE :



Preparation of PBAE



Preparation of PBAE

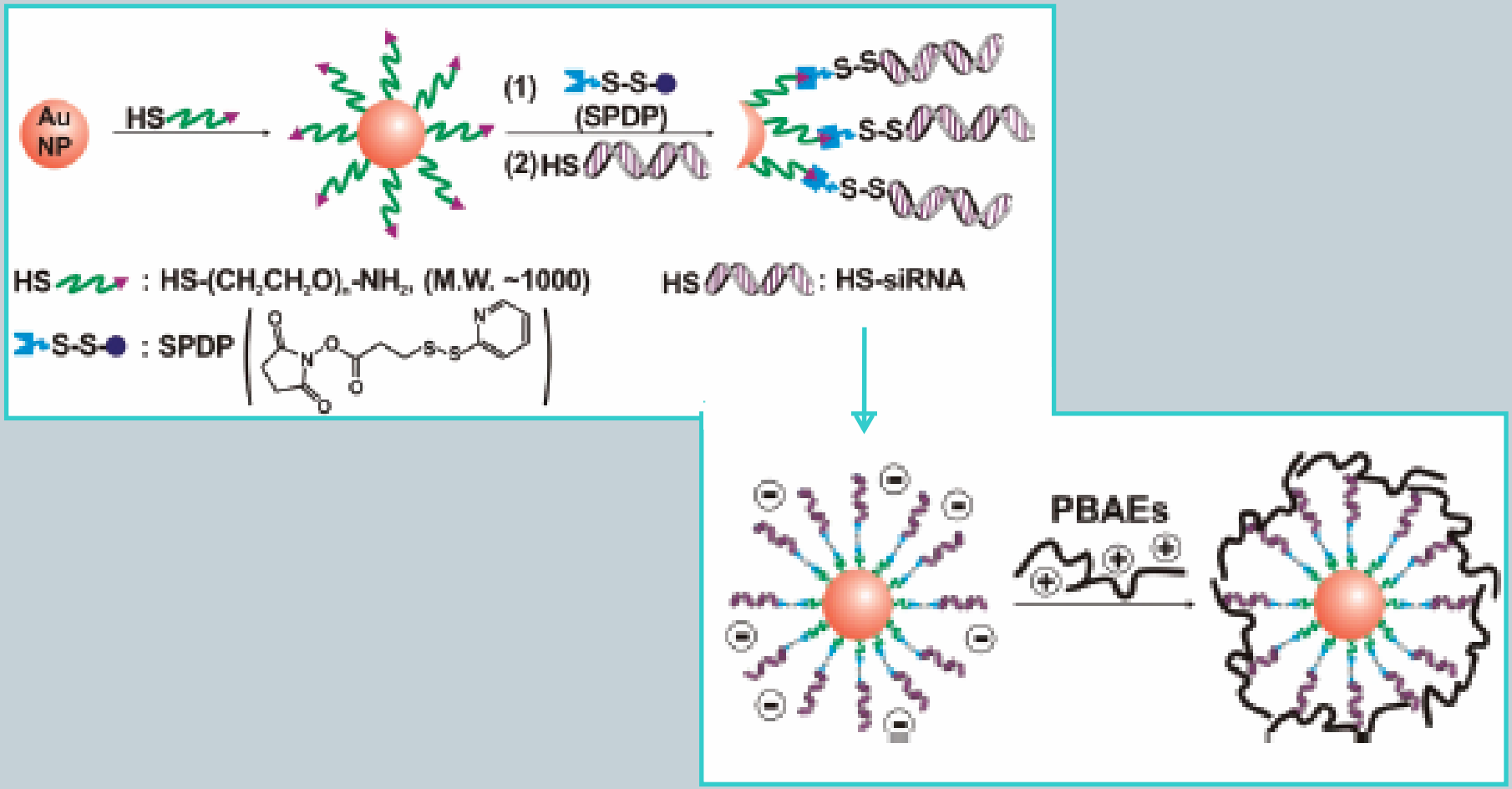
- Precipitation -

  End-modified polymers were precipitated by the addition of 10 volumes of diethyl ether and centrifugation at 2,500 rpm for 2 minutes.

- Washing -

  Polymers were washed twice and dried in a vacuum desiccator.

Preparation of Au-siRNA-PBAE nanoparticles



Preparation of Au-siRNA-PBAE nanoparticles

- Preparation was done in following steps,

dilution to

PBAE ⟶ 0.36 µg/µL

(100 µg/µL in DMSO) (In 25 mM acetate buffer,pH 5),

Rapid addition of siRNA-AuNPs (15 nM) to make,

↓

10.8 µg of PBAE and 300 fmol of AuNPs in 100 µL

(incubated for 10 min)

↓

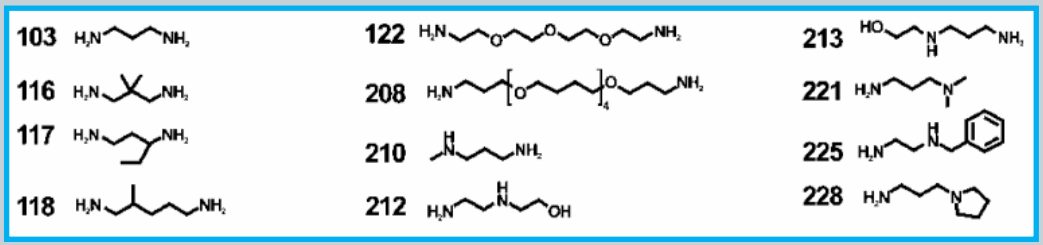
A

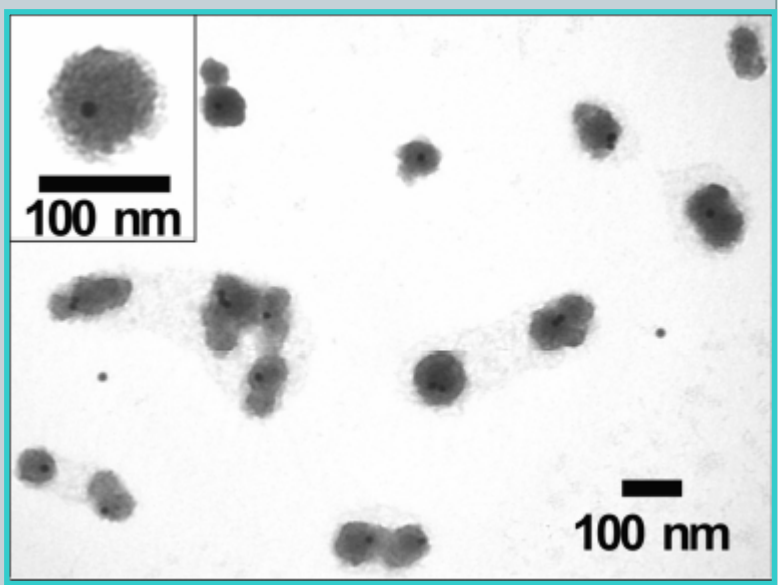
Preparation of Au-siRNA-PBAE nanoparticles

- Library of PBAEs was used to synthesize siRNA-AuNPs and then to screen for their ability to facilitate functional siRNA delivery, in vitro.
- ζ -potential measurements,

  siRNA-AuNPs= ca. -34 mV

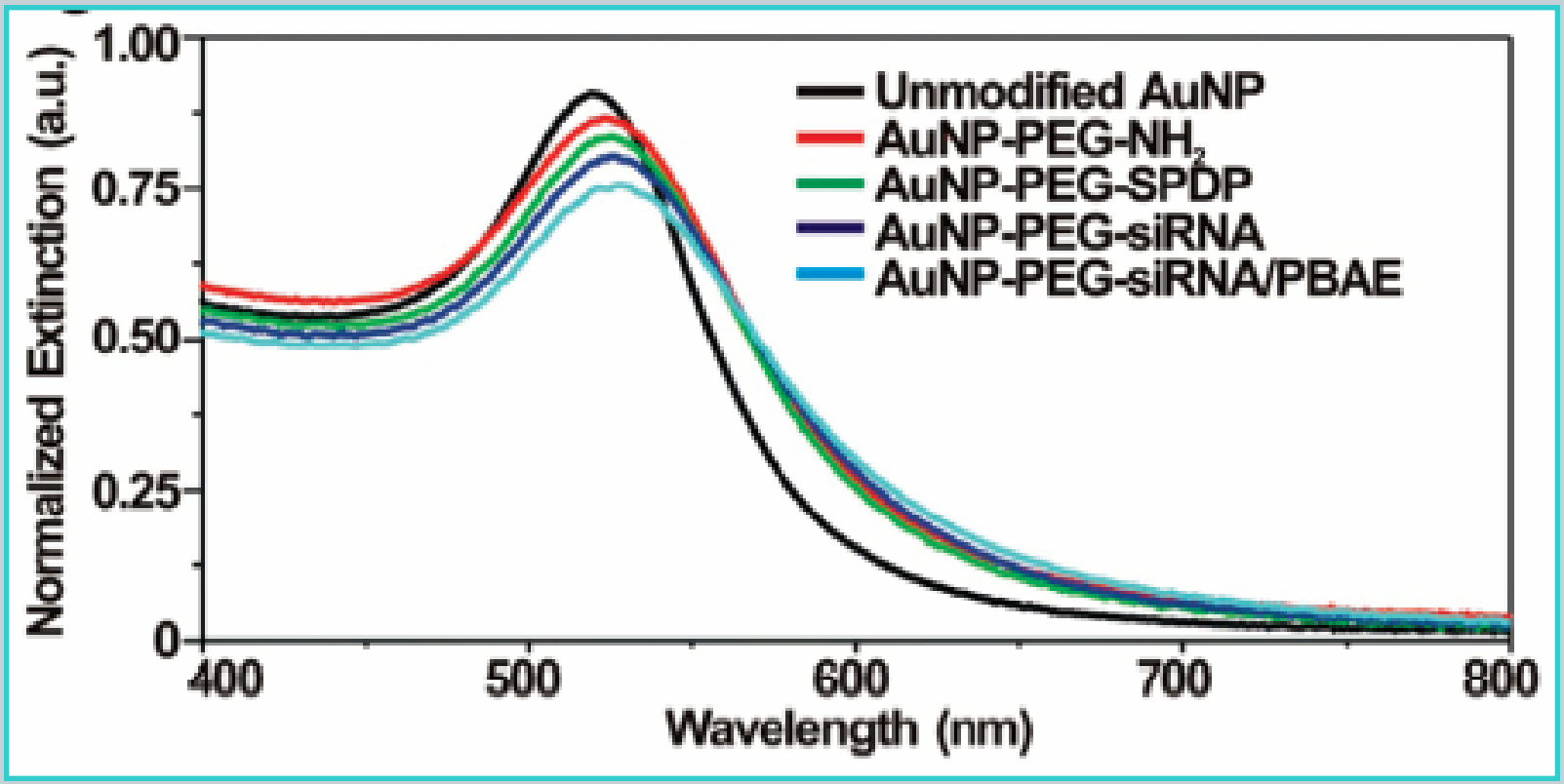
  PBAE-siRNA-AuNPs= ca. +13 mV





Preparation of Au-siRNA-PBAE nanoparticles

- UV-vis spectra taken during various synthetic steps,



Cellular transfection and gene knockdown

- HeLa cells were genetically engineered to express both firefly luciferase and Renilla luciferase.
- Then cells were allowed to adhere at 37 ^{o}C, 5 % CO_2 overnight in growth medium (10 % FBS and 90 % phenol red-free DMEM).

A + HeLa cells (in 100 µL well) → 4hr incubation → replaced by fresh growth medium (100 µL)

↓ 24hr incubation

↓ 37 ^{o}C, 5 % CO_2

Dual-GloTM Luciferase Assay

Protocol available at,

http://www.promega.com/tbs/tm058/tm058.html

Cellular transfection and gene knockdown

- Experimental Conditions for Lipofectamine 2000 (commercially available liposomebased delivery agent)

A = Lipo2000 (17.8 µL in 513 µL OptiMEM) and wait 5 min.

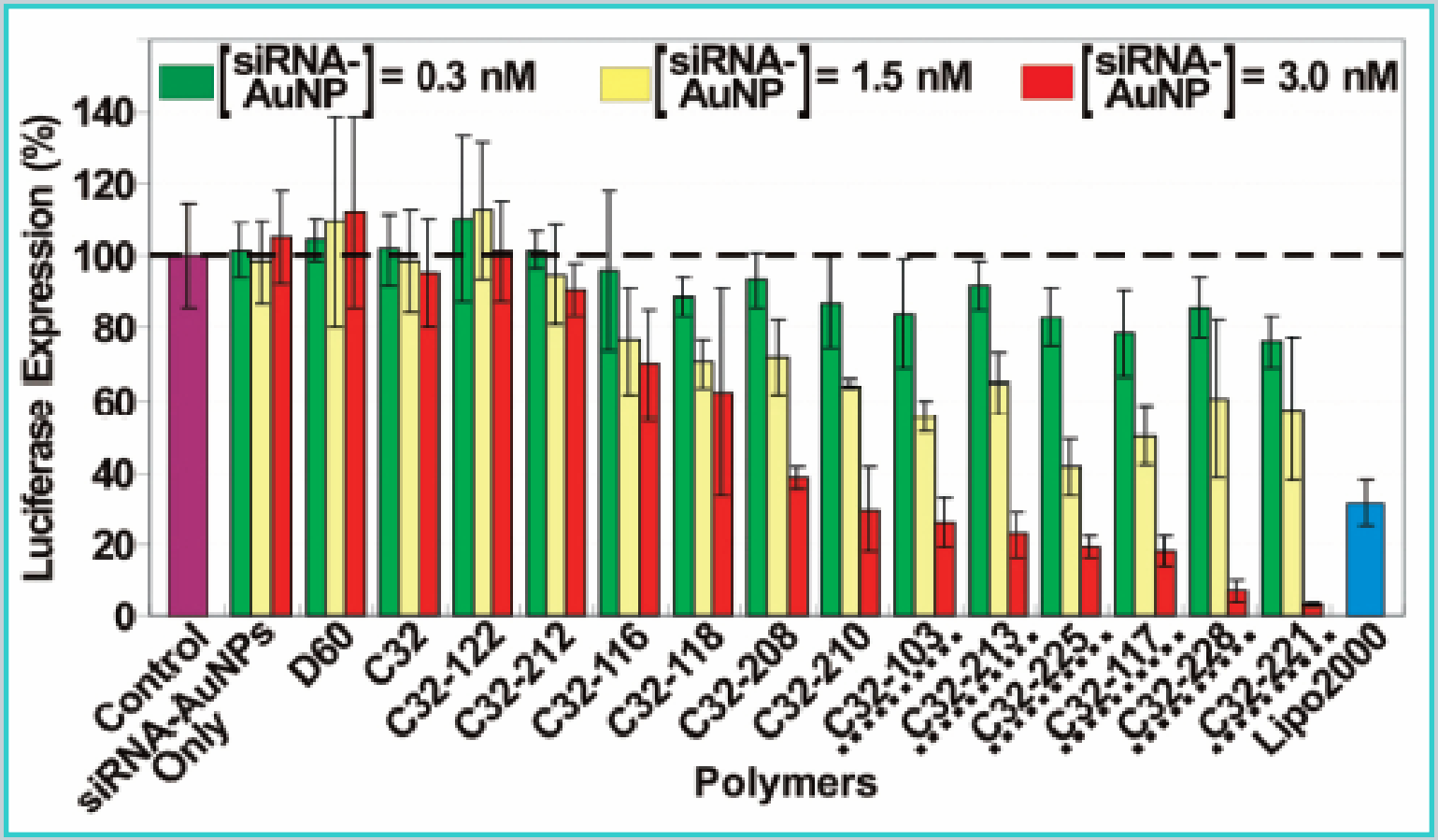
B = siRNA(0.28 mg/mL) diluted to 1.79 µL in 3.33 mL OptiMEM

C = Add 530 µL of B to A and wait 20 min.

D = Add 20 µL C to 130 µL of media of the positive control (The final [siRNA] = ~ 1 nM).

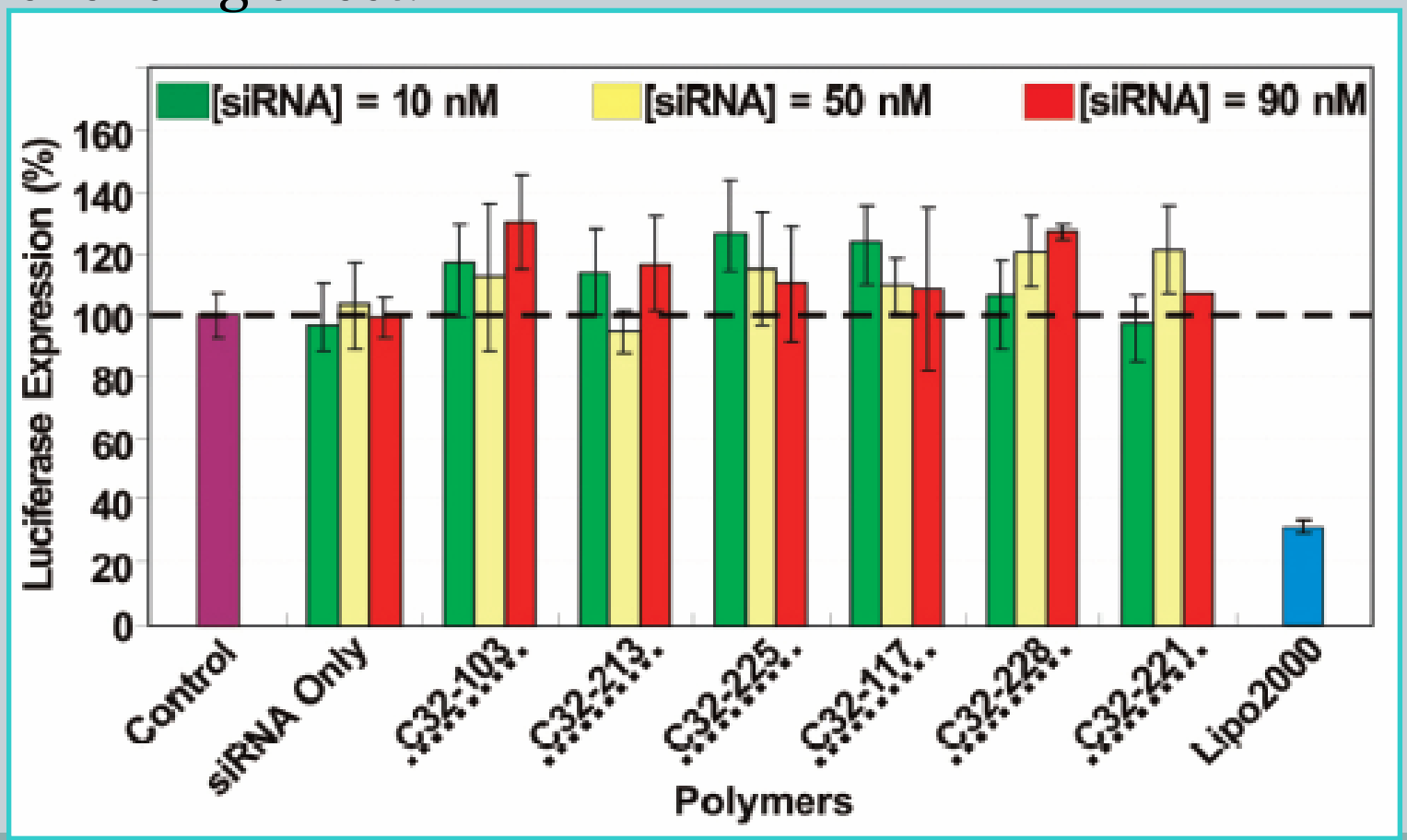
Results :

Control = Untreated cells



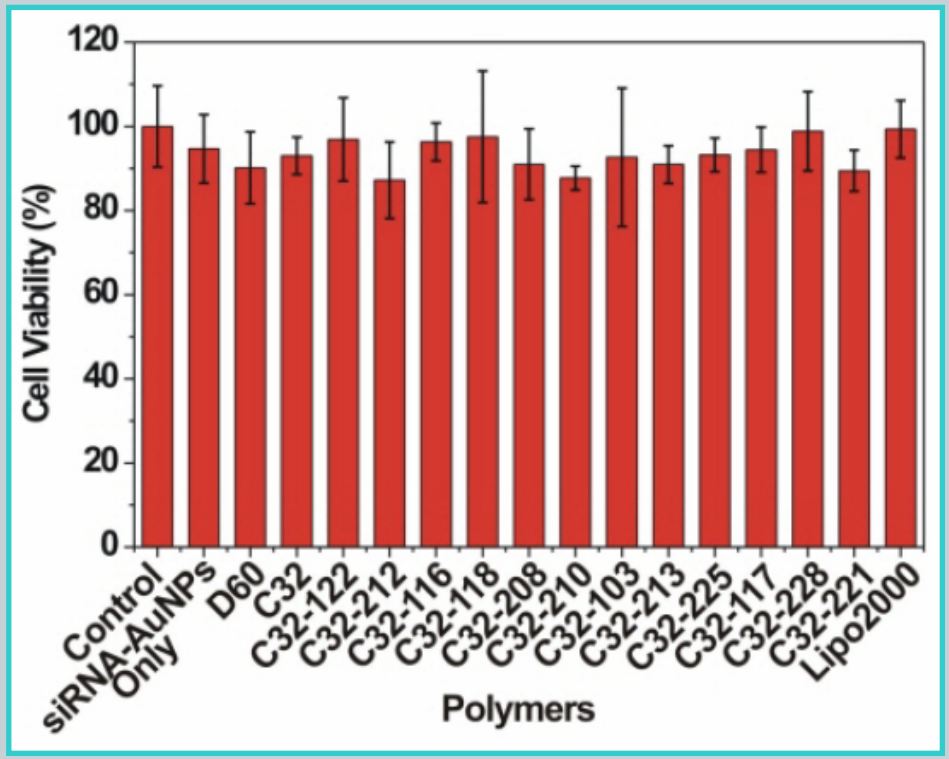
Results :

- Unassembled siRNA combined with PBAEs did not exhibit any silencing effect.



Results :

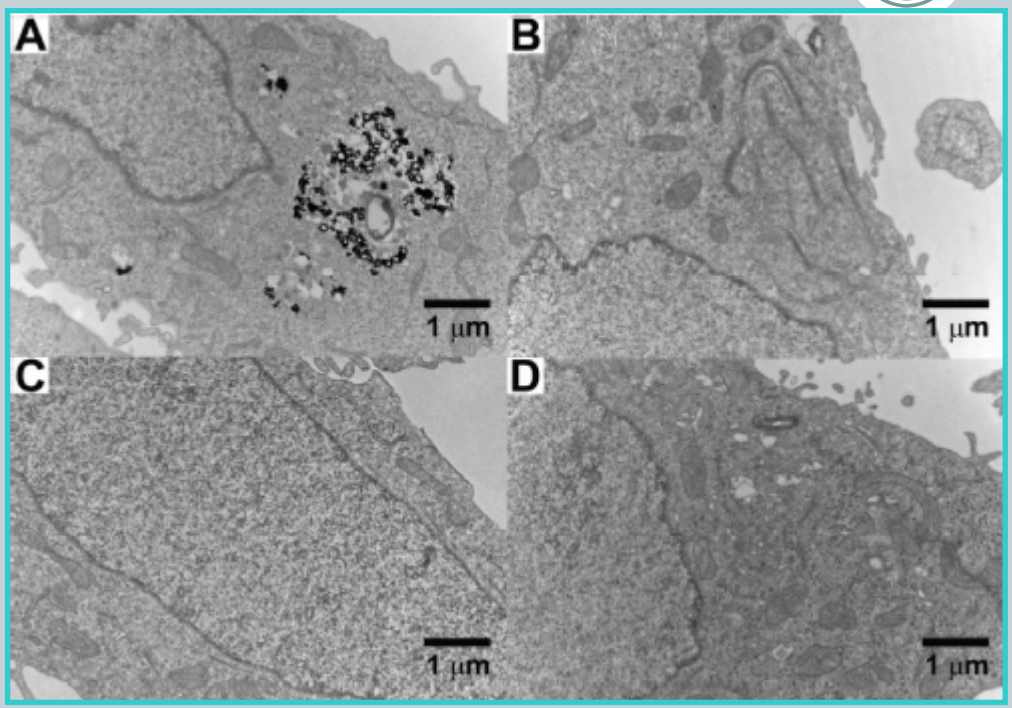
- Cell viability test was performed by Cell Titer96® AQueous One Solution Cell Proliferation Assay (Invitrogen)



- Protocol available at,

http://www.promega.com/tbs/tb245/tb245.html

Results :



TEM images of the HeLa cells exposed to 3 nM

(A) PBAE-siRNA-AuNPs

PBAE is C32-221,

(B) siRNA-AuNPs without PBAEs,

(C) unmodified AuNPs,

(D) no nanoparticles (control).

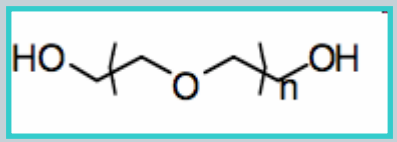
- The images were taken with a Tecnai G2 Spirit at HV 80 kV.

Conclusions :

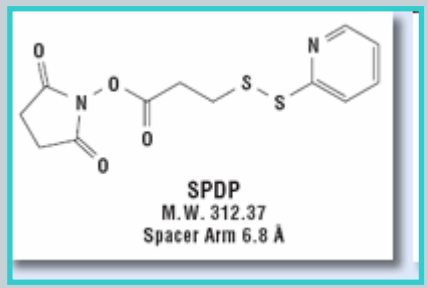
- PBAEs (C32-228 and 221) exhibit significantly better efficiency than the commercially available liposome based delivery agent (Lipofectamine2000, Invitrogen)
- No significant toxicity was observed when PBAE is used as siRNA delivery agent.
- PBAEs can be used as a delivery enhancer in gene/Drug delivery.
- Gold nanoparticles are essential as scaffolds for the development of some complex drug delivery vehicles.

Thank you

- $C_{2n+2}H_{4n+6}O_{n+2}$



- N-succinimidyl 3-(2-pyridyldithio) propionate



- DMSO is an important polar aprotic solvent. It is less toxic than other members of this class like HMPA. Because of its excellent solvating power, DMSO is frequently used as a solvent for chemical reactions involving salts.

- The sulfur center in DMSO is nucleophilic toward soft electrophiles and the oxygen is nucleophilic toward hard electrophiles.
- Opti-MEM® I Reduced Serum Media is ideal for use during cationic lipid transfections using our available list of transfection reagents.