Light–Controlled Protein Delivery Using Plasmonic Gold Nanoparticles

Name: Dorian Bruch
Major: Chemical Engineering
Mentor: Dean Morales
Faculty Advisor: Dr. Norbert Reich
Department: Chemistry/Biochemistry
Light–control is a solution to time–resolved studies of cellular processes

Protein

TAT

Protein image from: http://www.mrc-lmb.cam.ac.uk/mda201/projects/p53qm.html
Optogenetic proteins require substantial effort to create

Optogenetic proteins require substantial effort to create

Optogenetic protein (inactive) → $hv$ → Optogenetic protein (active)

Nanoparticles can provide a light-controlled release

Nanoparticle with Native Protein
Protein Released

Nanoparticles can provide a light-controlled release

Nanoparticle with Native Protein

hv

Protein Released
| Protein Purification | Nanoparticle Assembly | Delivery and Evaluation |
Protein Purification

Cells

Nanoparticle Assembly

Delivery and Evaluation
Protein Purification

Cells

Lysis

Crude Protein

Nanoparticle Assembly

Delivery and Evaluation
Protein Purification

Cells → Lysis → Crude Protein → Purification → Pure Protein
Protein Purification

Cells → Lysis → Crude Protein → Purification → Pure Protein

NTA → Nickel → Polyhistidine Tag
Nanoparticle Assembly

Dative Bond (semi-covalent)
Nanoparticle Assembly

Dative Bond (semi-covalent)

Protein Purification

Delivery and Evaluation

Polyhistidine Tag

NTA

Nickel
P53 Promoter Plasmid

ds Red

P21

Delivery and Evaluation
Delivery and Evaluation

P53 Promoter Plasmid

Nanoparticle Assembly

P21

ds Red

P53 Introduced

P53 Introduced
Protein Purification

P53 Promoter Plasmid

Nanoparticle Assembly

P21

Delivery and Evaluation

ds Red

P53 Introduced

Red Fluorescent Protein Produced
P53 EMSA
P53 EMSA

8 % NATIVE PAGE

0.42 mg/mL  0.84 mg/mL  4.2 mg/mL  8.4 mg/mL DNA Alone

Start  Finish
Purity of the P53

Ladder  1.6 mg/mL  3.3 mg/mL  0.8 mg/mL

53 kDa

12 % SDS PAGE
P53 Purification

Ladder

230 kDa
60 kDa
50 kDa
25 kDa
10 kDa

Start

12% SDS PAGE

Finish
P53 Purification

Start

12% SDS PAGE

Finish

Ladder  Crude

230 kDa
60 kDa
50 kDa
25 kDa
10 kDa

50 kDa
60 kDa
P53 Purification

Start

12% SDS PAGE

Finish

Ladder  Crude  Wash

230 kDa
60 kDa
50 kDa
25 kDa
10 kDa

Crude Ladder Wash
P53 Purification

Ladder  Crude  Wash  Concentration Increase

Start  Finish

12% SDS PAGE

230 kDa
60 kDa
50 kDa
25 kDa
10 kDa

Crude Ladder Wash Concentration Increase
Continued Efforts

• Establish control to show promoter is functional

• Delivery of p53 to cells with validated promoter
Acknowledgments

Dean Morales
Reich Laboratory
California Nanosystems Institute
Neural Research Institute
DNA Annealing

- Single strand only
- 500 ng
- 250 ng
- 125 ng
- 63 ng