WORK PERMIT

Department of Chemical and Biological Engineering

化學及生物工程學系

Project Title : Genetically encoded "Click" chemistry: A versatile tool for molecular engineering

Researcher(s) : Chan Yin Yung

Supervisor(s) : Prof. Fei Sun

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Signature of Approval :

Prof. Marshal LIU
Acting DSO

CBE work plan

No. WP 17047

Genetically encoded "Click" chemistry: a versatile tool for molecular engineering

Name of researcher: Chan, Yin Yung

Undergraduate Student

Name of supervisor: Fei SUN

Date: 2017-6-9

1. **General Information**

   Name of Researcher: Chan, Yin Yung

   Name of Project: Genetically encoded "Click" chemistry: a versatile tool for molecular engineering.

   Supervisor: Fei SUN

   Proposed location: Rm.6121, 6104, 6107, 6251 and 6/F Service Corridor

   Proposed start date: July 2017

2. **Experiment/Project Description**

   The synthesis of molecular systems with atomic precision for a specific application constitutes a fundamental challenge of molecular engineering. Development of the ability to construct complex protein architectures will provide a solution to overcome this obstacle. As revealed in our early work as well as others', genetically encoded click chemistry, e.g. SpyTag-SpyCatcher chemistry, which can covalently link two protein molecules together with high specificity and efficiency, enables precise control over macromolecule topology, synthesis of bioactive materials, and design of improved biocatalysts, thus representing a powerful tool for molecular engineering. The major goal of this project is to diversify genetically encoded click chemistry through a strategy empowered by both directed evolution and computation aided protein design. We anticipate that a toolbox that contains multiple orthogonal GECC pairs will greatly enrich the weaponry of molecular engineering.

3. **Equipment List**

   Rm. 6121:

   Refrigerator;
   Refrigerated Centrifuge;

   Room: 6104&6107:

   Balance,
   Analytical;
   pH meter;
   Refrigerator;
   Freezer;
   Biosafety cabinet;
   Autoclave;
   Microwave Oven;
   Water Bath;
   Multi-Therm Shaker;
   Auto-pipette p2, p20, p200, p1000;
   Auto-pipette 5ml, 10ml;
   Auto-pipette Aid;

Multi-channel Pipette;
Personal Microcentrifuge;
Bench-top Centrifuge;
Refrigerated Centrifuge;
Vortex;
Roto-Shake;
Orbital Shaker;
Incubator;
Shaking Water Bath;
Environmental Shaker;
CO_2 incubator;
Cryogenic Storage Tank;
Inverted Microscope CKX41;
Inverted Microscope TE2000-U;
Upright Biological Microscope BX41;
Sonifier cell disruptor;
Grinding Mill; Ultrasonic Water Bath;
Thermal Cycle 1;
Thermal Cycler 2;
Vacuum Concentrator – for DNA/RNA/Proteins;
Agarose Gel Electrophoresis Gel Tank and Power Supply;
Polyacrylamide Gel Electrophoresis and Blotting units;
Gel Documentation System

Rm.6251:
Zeiss Laser Scanning Confocal Microscope;
BTX Electroporation System; Centrifuge facilities

6/F Service Corridor:
Freeze Dryer;
Autoclave;
Centrifuge

Rm. 7119:
ARES Rheometer

4. Experimental Procedures

4.1 Bacterial strains, plasmids, and culture conditions

Grow *Escherichia coli* cultures in Luria Broth (LB) for cloning and in Terrific Broth (TB) for protein expression.

4.2 Recombinant protein expression and purification

(1)Grow *E. coli* strain harboring the appropriate plasmid at 37°C in TB medium to an optical density of 0.6 ~ 0.8 at 600 nm, and protein expression

was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 30°C.

(2)After 4 hours, cells are harvested and frozen at -80°C for at least 2 hours before protein purification.

(3) Purify proteins from the frozen cells on HisTrap columns following the column manufacturer's recommendations.

(4)Protein purity is greater than 95% as assessed by SDS-PAGE.

a) Reagents

30% N, N' Methylene-bis-acrylamide (stored in the dark)

Tris-HCl buffer (pH 8.8) and Tris-HCl buffer (pH 6.8)

10% ammonium persulfate solution (always should be prepared freshly)

TEMED

1X Tris-glycine-SDS Buffer (10X buffer diluted to 1X concentration prior use)

b) Procedure

1. Assemble the glass plate sandwich.
2. Add ammonium persulfate solution and TEMED last, mix carefully to avoid formation of bubbles.
3. Important Note. Polymerization begins as soon as ammonium persulfate solution is added to the mixture, so all subsequent actions must be performed promptly.
4. Pour the gel solution between the glass plates with a pipette, leave about 1/4 of the space free for the stacking gel. Carefully cover the top of the resolving gel with 50% isopropanol, 0.1% SDS solution or water, and wait until the resolving gel polymerizes (~30 min). A clear line will appear between the gel surface and the solution on top when polymerization is complete.
5. Discard the water, isopropanol or SDS solution. Wash gently with double-distilled water.
6. Pour the stacking gel solution (prepared as described above, add ammonium persulfate solution and TEMED last) carefully with a pipette to avoid formation of bubbles.
7. Insert combs. Allow the gel to polymerize for at least 60 min.
8. Remove combs carefully. Put the gel into the electrophoresis tank, fill the tank (bottom and top reservoirs) with fresh 1X Tris-glycine-SDS Buffer, make sure that the gel wells are covered with the buffer.
9. Load protein ladder/marker and probes.
10. Set an appropriate voltage and current depending on how many gels you run. Increase the power when the dye front reaches the running gel.
11. Stop the electrophoresis run when the dye front reaches the bottom of the gel. Disassemble the gel sandwich and proceed with gel staining or Western blot procedures.

(5)The resulting proteins are subject to extensive dialysis against distilled water (4 L x 7) at 4°C, flash frozen in liquid N_2 and lyophilized by the freezer

dryer in 6/F Service Corridor at -80°C for 2 days. 2-Mercaptoethanol can be used as a biological antioxidant by scavenging hydroxyl radicals. The protein can be marked by Bromophenol Blue. Determine the total level of protein in a solution by using Bicinchoninic acid.

(6) Store lyophilized proteins at -80°C before use.

4.3 Preparation of Spy networks.

(1) Dissolve lyophilized proteins in distilled water to make 12 wt% solutions.

(2) To form the Spy network, AAA* (12 wt%) and BB** (12 wt%) are manually mixed at a molar ratio of 2:3 in an Eppendorf tube to initiate gelation at room temperature.

(3) Rheology measurement. The technician, Pauline LEUNG, in CBME will train me to follow standard operating procedure. Standard operating procedure: First, zero the 25mm parallel plate fixture. Then load the sample. And then run the test.

4.4 Erosion-rate measurements

Prepare protein mixtures (volume, 50 μL) in Eppendorf tubes as described above. Add distilled water(1.2 mL) to immerse the sample. Take aliquots (25 μL)at different time points and stored at -20°C for subsequent analysis. Determine the amount of protein released into water using Pierce BCA protein assays.

4.5 Encapsulation of neurons

(1) Coat microtiter and multiwall plates with Poly-D-lysine (PDL) overnight in 37°C.

(2) Wash PDL by ddH_2O 5 times, air dry in hood with UV on.

(3) Coat laminin, 1:100 in Nutrient Broth (NB), in 37°C for over 1 hour.

(4) Dissect DRG neurons (provided by collaborators from the Dr. Liu Kai group at Division of Life Science of HKUST) into 480μl ice-cold HBSS (1.5 ml/tube).

(5) Add 25μl 0.125% collagenase in each tube, in 37°C for 1.5-2 hours.

(6) Discard supernatants, add 300μl 0.25% Trypsin in 37°C for less than 30 minutes.

(7) Discard supernatants, add 100μl medium (10% FBS, DMEM, and P/S).

(8) Resuspend the neurons with 15μl BB solution (12 wt%) and place it in a 12mm petri dish. The AAA solutions (4.5μl, 12 wt%) are manually mixed with the cell suspension to initiate gelation.

(9) Cure the gels at 37°C for 30 minutes. After gelation, add 1mL medium to cover the sample. Incubate the cells at 37°C with 5% CO_2. Change the medium every 24 hours.

(10) 2-4 days later, immune staining.

4.6 Immunocytochemistry and fluorescence confocal microscopy

(1) Encapsulate neurons in Spy networksfor 4 days. After removal of the medium, wash the gels with 1 mL PBS and fixed with 4% Paraformaldehyde (PFA) for 25 minutes at 37°C.
(2) Neuronsare permeabilized with 100% methanol for 3 minutes at room temperature, washed three times with fresh PBS, and blocked with 10% Normal Goat Serum (NGS) in PBS overnight at 4°C.
(3) Neuronsare incubated with mouse anti-Oct4 antibody (BD Biosciences) at 1.25 μg/mL final concentration in 10% NGS in PBS overnight, washed three times with PBS and incubated for 2 hours at room temperature with goat anti-mouse IgG Alexa Fluor-488 conjugate (Invitrogen) secondary antibody at 1:2000 dilution in PBS.
(4) Remove the secondary antibody solution by three washes with PBS.
(5) Neuronsare labeled with DAPI (Life Technologies) at 300 nM and rhodamine phalloidin(Life Technologies) at 100 nM for 1 hour, and washed three times with PBS.
(6) Cover the samples with 10% glycerol in PBS for imaging purposes.
(7) Fluorescence confocal images are obtained on a Zeiss LSM 510 microscope equipped with a 760 nm laser for two-photon excitation of DAPI. Images are analyzed inImageJ.

AAA*: Protein expressed by constructed gene *SpyTag-ELP-SpyTag-ELP-SpyTag*

BB**: Protein expressed by constructed gene *SpyCatcher-ELP-SpyCatcher*

5.PROCEDURE TEMPLATE

Experimental Procedure No.	Experimental Procedure Description	Scale (Mass/Volume)	Location (Fumehood, benchtop,etc)	Method New or Existing
1.1	Grow *Escherichia coli* cultures in Luria Broth (LB) for cloning and in Terrific Broth (TB) for protein expression.	LB: 10g TB: 900mL	Benchtop	Existing
2.1	Grow *E. coli* strain harboring the appropriate plasmid at 37°C in TB medium to an optical density of 0.6 ~ 0.8 at 600 nm, and protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 30°C.	TB: 900mL IPTG: 0.24g	Benchtop	Existing
2.2	After 4 hours, cells are harvested and frozen at -80°C for at least 2 hours before protein purification.		Benchtop	Existing
2.3	Purify proteins from the frozen cells on HisTrap columns following the column manufacturer's recommendations. Determine the total level of protein in a solution by using Bicinchoninic acid.	Bicinchoninic acid: 1mL	Benchtop	Existing
2.4	Protein purity is greater than 95% as assessed by SDS-PAGE (prepared by N,N' methylene-bis-acrylamide, ammonium persulphate, TEMED, HCl). The protein can be marked by Bromophenol Blue.		Benchtop	Existing
2.5	The resulting proteins are subject to extensive dialysis against distilled water (4 L x 7) at 4°C, flash frozen in liquid N_2 and lyophilized at -80°C for 2 days.	DI water: 28L Liquid N_2: 50mL	Benchtop	Existing
2.6	Store lyophilized proteins at -80°C before use. Use biological antioxidant regent 2-Mercaptoethanol to reduce disulfide bonds.	2-Mercaptoethanol: 2µl	Benchtop	Existing
3.1	Dissolve lyophilized proteins in distilled water to make 12 wt% solutions.	DI water: 1mL	Benchtop	Existing

3.2	To form the Spy network, AAA (12 wt%) and BB (12 wt%) are manually mixed at a molar ratio of 2:3 in an Eppendorf tube to initiate gelation at room temperature.		Benchtop	Existing
4.1	Prepare protein mixtures (volume, 50 μL) in Eppendorf tubes as described above. Add distilled water(1.2 mL) to immerse the sample. Take aliquots (25 μL)at different time points and stored at -20°C for subsequent analysis. Determine the amount of protein released into water using Pierce BCA protein assays.	DI water: 1.2 mL	Benchtop	Existing
4.3	Zero the 25mm parallel plate fixture. Then load the sample. And then run the test.	Sample: 2mL	Benchtop	Existing
5.1	Coat microtiter and multiwall plates with Poly-D-lysine (PDL) overnight in 37°C.	Compressed Nitrogen	Biosafety Cabinet (BSC)	Existing
5.2	Wash PDL by ddH_2O 5 times, air dry in hood with UV on.	DI water: 50mL	BSC	Existing
5.3	Coat laminin, 1:100 in Nutrient Broth (NB), in 37°C for over 1 hour.	Laminin: 1g	BSC	Existing
5.4	Dissect DRG neurons into 480μl ice-cold HBSS (1.5 ml/tube).		Benchtop	Existing
5.5	Add 25μl 0.125% collagenase in each tube, in 37°C for 1.5-2 hours.	Collagenase: 0.125%, 25μl	BSC	Existing
5.6	Discard supernatants, add 300μl 0.25% Tripsin in 37°C for less than 30 minutes.	Tripsin: 0.25%, 300μl	BSC	Existing
5.7	Discard supernatants, add 100μl medium (10% FBS, DMEM, and P/S).	Medium: 100μl	BSC	Existing
5.8	Resuspend the neurons with 15μl BB solution (12 wt%) and place it in a 12mm petri dish. The AAA solutions (4.5μl, 12 wt%) are manually mixed with the cell suspension to initiate gelation.		BSC	Existing

5.9	Cure the gels at 37°C for 30 minutes. After gelation, add 1mL medium to cover the sample. Incubate the cells at 37°C with 5% CO_2. Change the medium every 24 hours.		BSC, Incubator	Existing
5.10	2-4 days later, immune staining.		Benchtop	Existing
6.1	Encapsulate neurons in Spy networksfor 4 days. After removal of the medium, wash the gels with 1 mL PBS and fixed with 4% Paraformaldehyde (PFA) for 25 minutes at 37°C.	PBS: 1mL PFA: 1mL	BSC	Existing
6.2	Neuronsare permeabilized with 100% methanol for 3 minutes at room temperature, washed three times with fresh PBS, and blocked with 10% Normal Goat Serum (NGS) in PBS overnight at 4°C.	Methanol: 1mL NGS: 1mL	BSC	Existing
6.3	Neuronsare incubated with mouse anti-Oct4 antibody at 1.25 µg/mL final concentration in 10% NGS in PBS overnight, washed three times with PBS and incubated for 2 hours at room temperature with goat anti-mouse IgG Alexa Fluor-488 conjugatesecondary antibody at 1:2000 dilution in PBS.	NGS: 1mL PBS: 5mL	BSC	Existing
6.4	Remove the secondary antibody solution by three washes with PBS.		BSC	Existing
6.5	Neurons are labeled with DAPI at 300 nM and rhodaminephalloidin(Life Technologies) at 100 nM for 1 hour, and washed three times with PBS.	DAPI: 30nmol	BSC	Existing
6.6	Cover the samples with 10% glycerol in PBS for imaging purposes.	Glycerol: 100µl PBS: 1mL	BSC	Existing
6.7	Fluorescence confocal images are obtained on a Zeiss LSM 510 microscope equipped with a 760 nm laser for two-photon excitation of DAPI. Images are analyzed inImageJ.		Benchtop	Existing

6.

HAZOP Template

Hazard and Operability Analysis

Activity:

NO	HAZARD	HAZARD EFFECT	SEVERITY	PROBABILITY	RISK	MINIMISE RISK BY	RESIDUAL RISK
1.1	N/A						
2.1	IPTG	Higher concentration may become toxic to the cell.	L	L	L	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
2.2	N/A						
2.3	N/A						
2.4	Acrylamide	Neuron-toxin, cryogenic injury	M	L	M	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
	Ammonium persulphate	Ammonium persulfate has low dermal toxicity and is moderately toxic when ingested.	L	L	L	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
	TEMED	Toxic by ingestion, Corrosive.	M	L	M	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
	HCl	Corrosive to the eyes, skin, and mucous membranes.	M	L	M	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
	Bromophenol Blue	May cause respiratory tract irritation. May be harmful if absorbed through skin.	L	L	L	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L

2.5	Contact with liquid N_2	Careless handling of liquid nitrogen may result in cold burns.	L	L	L	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
2.6	2-Mercaptoethanol	2-Mercaptoethanol forms adducts with free cysteines.	L	L	L	Lab coat, gloves Goggle, lab coat, cryogenic gloves	L
3.1	N/A						
3.2	N/A						
4.1	N/A						
4.3	Overload the transducer	Damage the transducer	M	M	M	Follow the instruction	L
	Run the test	Overload the transducer	M	M	M	Follow the instruction	L
5.1	Ethanol	Flammable	L	L	L	Goggle, lab coat, cryogenic gloves	L
5.2	N/A						
5.3	N/A						
5.4	N/A						
5.5	N/A						
5.6	N/A						
5.7	N/A						
5.8	N/A						
5.9	N/A						
5.10	N/A					Lab coat,gloves, ventilate, handle in fumehood	
6.1	PFA	As a formaldehyde releasing agent, paraformaldehyde is a suspected carcinogen.	M	L	M	Lab coat,gloves, ventilate, handle in fumehood	L
6.2	Methanol	Flammable, Acute toxicity via inhalation, dermal and oral	M	M	M		L
6.3	N/A						
6.4	N/A					Lab coat,gloves, ventilate, handle in	

6.5 6.6 6.7	Rhodamine Phalloidin N/A N/A	Phalloidin is a bicyclic peptide belonging to a family of toxins.	M	L	M	fumehood	L
FINAL ASSESSMENT:						OVERALL RISK:	L

Remark: Severity–L=Low (Minor injuries, first aid); M=Medium(Hospitalization, medical leave); H=High(Serious injuries, fatality)

Probability–L=Low (Unlikely); M=Medium (Possible); H=High (Very Likely)

Note: Severity x Probability = Risk [eg. LxL=L; LxM=M;LxH=H; HxM=H; the product follows the higher severity or probability]

Higher Risk requires extensive risk minimization procedures

7. Operating Conditions

Neurons are cultured in incubator at 37 °C, 5% CO_2;
Other procedures could be operated under normal temperature and pressure.
Autoclave, high temperature and pressure.

8. Service List

Deionized water, electricity (220V, 50Hz)

9. Chemical List

Chemical	Quantity per experiment	MSDS attached
Ethanol	20ml	Yes
Acetone	50ml	Yes
Methanol	50ml	Yes
Dulbecco's phosphate buffered saline (PBS)	5mL	Yes
Normal goat serum (NGS)	2mL	Yes
Fetal bovine serum (FBS)	2mL	Yes
Trypsin	3mL	Yes
Luria Broth (LB)	10g	Yes
Terrific Broth (TB)	900mL	Yes
Isopropyl β-D-1-thiogalactopyranoside (IPTG)	0.24g	Yes
Poly-D-lysine (PDL)	0.1mg/ml, 50μL	Yes
Laminin	1mg	Yes
Nutrient Broth (NB)	2mL	Yes
HBSS	1.5mL	Yes
Collagenase	0.125%, 25μL	Yes
Dulbecco's Modified Eagle's Medium (DMEM)	3mL	Yes
Penicillin/Streptomycin Solution (P/S)	1mL	Yes
Paraformaldehyde (PFA)	3mL	Yes
Mouse anti-Oct4 antibody	10 μL	Yes
Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) Antibody	10 μL	Yes
4',6-diamidino-2-phenylindole(DAPI)	10 μL	Yes
Rhodaminephalloidin	10 μL	Yes
BCA Protein Assay Reagent (bicinchoninic acid)	1mL	Yes
N,N' Methylene-bis-acrylamide	30%, 200ml	Yes
Sodium dodecyl sulphate (SDS)	10%, 10ml	Yes
Ammonium persulphate	10%, 1ml	Yes
Tetramethylethylenediamine (TEMED)	20 μL	Yes
Tris(hydroxymethyl)aminomethane (Tris)	100ml	Yes

Hydrochloric acid (HCl)	20ml	Yes
2-Mercaptoethanol	1μL	Yes
Bromophenol Blue	0.5%, 20ml	Yes

10. Biological Agents List

Biological Agents	Biological Safety Level
Non-pathogenic Escherichia coli	Biosafety Level 1 (BSL-1)
Rat Neurons	Biosafety Level 1 (BSL-1)

11. Summary of Relevant Hazards and Incompatibilities

Chemical or Biological Agents	Concentration	Summary of Relevant Hazards	Incompatibilities
Ethanol	75%	Flammable liquids	Alkali metals, Ammonia, Oxidizing agents, Peroxides
Acetone	1×	Flammable liquids, Eye irritation, Specific target organ toxicity-single exposure	Bases, Oxidizing agents, Reducing agents, Phosphorous oxychloride
Methanol	1×	Flammable liquids, Acute toxicity, Inhalation; Acute toxicity, Dermal; Acute toxicity, Oral	Acid chlorides, Acid anhydrides, Oxidizing agents, Alkali metals, Reducing agents, acids
Dulbecco's phosphate buffered saline (PBS)	1×	Not a hazardous substance or mixture not classified as dangerous.	Strong oxidizing agents
Glycerol	10%	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Normal goat serum (NGS)	10%	Not a hazardous	Strong oxidizing agents

		substance or mixture Not a hazardous substance or mixture.	
Fetal bovine serum (FBS)	10%	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Trypsin	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Luria Broth (LB)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Terrific Broth (TB)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Isopropyl β-D-1-thiogalactopyranoside (IPTG)	1×	Acute toxicity, Inhalation Acute toxicity, Dermal Acute toxicity, Oral Very toxic by inhalation, in contact with skin and if swallowed.	Strong oxidizing agents
Poly-D-lysine (PDL)	1×	Not a hazardous substance or mixture Not a	Strong oxidizing agents

		hazardous substance or mixture.	
Laminin	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Nutrient Broth (NB)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
HBSS	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Collagenase	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Dulbecco's Modified Eagle's Medium (DMEM)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Penicillin/Streptomycin Solution (P/S)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Paraformaldehyde (PFA)	1×	Acute toxicity, Inhalation Acute toxicity,	H_2SO_4, HNO_3, caustics, ammonia, amines, alcanoamines.

		Dermal Acute toxicity, Oral Very toxic by inhalation, in contact with skin and if swallowed.	
Mouse anti-Oct4 antibody	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) Antibody	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
4',6-diamidino-2-phenylindole (DAPI)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Rhodamine phalloidin	1×	Acute toxicity, Inhalation; Acute toxicity, Dermal; Acute	Strong oxidizing agents
Bicinchoninic acid	1×	Acute toxicity, Inhalation; Acute toxicity, Dermal; Acute	Strong oxidizing agents
N,N' Methylene-bis-acrylamide	30%	Acute toxicity, Oral Very toxic by inhalation, in contact with skin and if swallowed.	Strong oxidizing agents

Sodium dodecyl sulphate (SDS)	10%	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Ammonium persulphate	10%	Acute toxicity, Inhalation; Acute toxicity, Dermal; Acute	Strong oxidizing agents
Tetramethylethylenediamine (TEMED)	1×	Acute toxicity, Oral Very toxic by inhalation, in contact with skin and if swallowed.	Strong oxidizing agents
Tris(hydroxymethyl)aminomethane (Tris)	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Hydrochloric acid (HCl)	1×	Highly corrosive, Acute toxicity, Oral Very toxic by inhalation, in contact with skin and if swallowed.	Strong alkaline
2-Mercaptoethanol	1×	Not a hazardous substance or mixture Not a hazardous substance or mixture.	Strong oxidizing agents
Bromophenol Blue	0.5%	Acute toxicity, Inhalation; Acute toxicity, Dermal;	Strong oxidizing agents

12. Waste List

Biological waste: Cells, used cell culture medium (pH~7.0),

Disposable Equipment: pipette tips, culture flasks, tubes, *etc.* The equipment may take biologically active materials, for example, cells.

Waste above subject to the standard protocol of biological waste including bleaching and autoclaving.

13. Assessment of Significant Risks

(1) When operating with IPTG, PFA, and rhodaminephalloidin, lab coat and gloves are required. Most of the procedure should be carried out in a biosafety cabinet. Personal protection equipment such as lab coat, gloves, goggles and respiratory protection are necessary, and operated in safety cabinet when handling with the chemicals. It should be stored in cool place. Keep container tightly closed in dry and well-ventilated place.

(2) Methanol and ethanol are volatile liquid which is hazard by breathing in. Ethanol, acetone and methanol are flammable liquid and might cause fire. When using these chemicals, mask is suggested. Moreover, keep them away from fire. Avoid inhaling methanol for it may cause blindness and do harm to liver and kidney. Researcher should wear goggles when handling with acetone, for it is eye irritant.

(3) Biological active cells are the main biological hazard material. They should be decontaminated in autoclave after use.

14. Safety Precautions

(1) Personal protection

Researchers in this project should wear lab coat and proper gloves against direct contact of hazard substance.

(2) Training

Before researches carry out any experiment in bioengineering laboratory, they must be trained proper operation of equipment and proper use of the chemical and biological substance in the experiment. Moreover, safety training is also necessary for researchers, which provides an introduction of hazard agents, how to disposal waste and how to deal with emergency situations. Researchers are required to pass all the relevant tests in order to have access to the bioengineering labs.

15. Action in Case of Abnormal Emergency Situations

(1) Service failure

In case of the failure and damage of the experimental instrument, switch it off and cut the power supply immediately and then inform the technician before taking any further actions.

(2) Fire or explosion

Incase of a fire or explosion, the room should be evacuated immediately and

the danger alarm should be activated in order to inform the HESO and the campus security or inform them by dialing 8999 at a safe place.

(3) Loss of containment or spillage

For small spillage, inform the technician immediately and clean up the area carefully adhering to the corresponding safety procedures.

For large spillage or loss of containment, the room should be evacuated immediately and the danger alarm should be activated in order to inform the HESO and the campus security or inform them by dialing 8999 at a safety place.

(4) Other possible abnormal situation

N/A

16. CBME Risk Assessment Audit Declaration