UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: BICX04 CURRENT ADVANCES IN BIOTECHNOLOGY
SEMESTER	: Second
EXAM	: SSA January 2020

DATE	: January 2020	**SESSION**	: 08:00-11:00
ASSESSOR(S)	: PROF LA PIATER & Guest Lecturers		
MODERATOR	: DR L STEENKAMP		
DURATION	: 3 HOURS	**MARKS**	: 100

NUMBER OF PAGES: 5 PAGES (including this page)

INSTRUCTIONS:

1. Answer ALL THE QUESTIONS.
2. Number your answers clearly

QUESTION 1: Genes and Genomes **[10]**

Humans have tens of thousands of genes, and the development of DNA microarrays by Patrick O. Brown, Joseph DeRisi, David Botstein, and colleagues in the mid-1990s has led to many applications. Describe how microarrays work, and discuss how it has been used in cancer diagnosis and treatment thus far? (10)

QUESTION 2: Yeast Biotechnology **[10]**

2.1 Briefly discuss how a yeast cell may be engineered into (i) producing useful plant secondary metabolites and (ii) "humanizing" (authentic glycosylation) proteins. (7)

2.2 Briefly discuss the application of each of the following techniques:

2.2.1 TAR (1)

2.2.2 Yeast-two hybrid system (2)

QUESTION 3: Plant Biotechnology **[10]**

What are the genetic engineering strategies to create the following traits in transgenic crops. Where applicable, give an example of each:

a) Herbicide tolerance (5)

b) Virus resistance (5)

QUESTION 4: Molecular Diagnostics **[10]**

"HIV RNA (viral load) and CD4 T lymphocyte (CD4) cell count are the two surrogate markers of antiretroviral treatment (ART) responses and HIV disease progression that have been used for decades to manage and monitor HIV infection. Viral load is a marker of response to ART. A patient's pre-ART viral load level and the magnitude of viral load decline after initiation of ART provide prognostic information about the probability of disease progression. The key goal of ART is to achieve and maintain

durable viral suppression. Thus, the most important use of the viral load is to monitor the effectiveness of therapy **after** initiation of ART."

4.1. Design an experiment in which you measure the viral load of HIV in blood plasma of patients that have received ART treatment. (7)

4.2. "The co-circulation of different HIV types and groups can lead to dual infections and recombinants, which hinder diagnosis and therapeutic management". Suggest and justify ways in which you would modify your experiment approach in 4.1 in order to detect and measure different HIV types and groups HIV in ARV-treated vs. pre-ART treated patients. (3)

QUESTION 5: Biotechnology-based Drug Discovery **[10]**

5.1. State the drug discovery process in sequence and indicate how long does this process take on average. (6)

5.2. What considerations should be made when selecting a suitable screening assay? (3)

5.3. What is the parameter during lead identification that measures the efficacy of the lead compound? (1)

QUESTION 6: Vaccines **[10]**

List 4 differences between OPV and IPV polio vaccines, and the reason/s why we are slowly withdrawing OPV.

QUESTION 7: Stem Cells **[10]**

7.1 In stem cell-based therapies, the cells that are used within the therapy can be sourced in two different methods, either autologous or allogeneic. What is meant by these two terms? (2)

7.2 CAR-T therapy is a combination of genome engineering and cellular replacement therapy that is at the forefront of precision medicine. Discuss the concept behind the therapy including advantages and disadvantages of the treatment. (8)

QUESTION 8: Biofuels **[10]**

8.1 Various feedstocks are used to produce biofuels. Using corn as a feedstock, explain in detail the two processes employed for corn processing.

(10 X ½ = 5)

8.2 Biotechnology is the exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms to produce desired products. Using any two genetic manipulation approaches, explain how you will enhance both cellulose break down and sugar fermentation. Use 1 approach for cellulose and 1 approach for sugar fermentation (5)

QUESTION 9: Crystallography **[15]**

9.1 In your crystallography practical in July 2019, you attempted purification of two protein components that together were meant to crystallize.

9.1.1 The target protein MAGEB1 has a molecular weight of 29kDa, and the nanobody has 13k kDa. After purification, you have 80ul of MAGEB1 at a concentration of 5 mg/ml, and 130ul of the nanobody at 3 mg/ml. What volumes of each solution must you mix together to yield a solution where the nanobody is in 2x molar excess? Show your calculations. (2)

9.1.2 Explain the role of the Nickel in the IMAC purification. (1)

9.1.3 During purification, after adding the crude lysate to the IMAC column and doing several wash steps, you had to do a pre-elution with low

concentrations of imidazole. Explain why this was necessary, and how the imidazole addresses this. (3)

9.2 Apart from intrinsic properties of a protein, describe aspects of the experiment that are in your control that can improve chances of getting the protein to crystallize. (3)

9.3 Discuss how entropy drives both micelle formation and therefore protein folding. (3)

9.4 Describe a general strategy for achieving larger crystals simply by changing concentrations of crystallization components, and explain the principle; sketch a schematic phase diagram if it simplifies your answer. (3)

QUESTION 10: Application of knowledge in project **[5]**

During your individual BICX00 research project, you made use of “omics” (genomics, proteomic, metabolomics, *etc.*) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.

MEMO

QUESTION 1: Genes and Genomes [10]

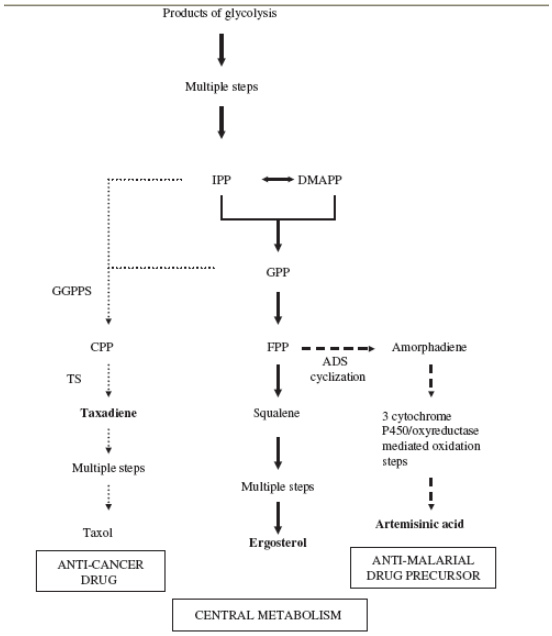
Humans have tens of thousands of genes, and the development of DNA microarrays by Patrick O. Brown, Joseph DeRisi, David Botstein, and colleagues in the mid-1990s has led to many applications. Describe how microarrays work, and discuss how it has been used in cancer diagnosis and treatment thus far? (10)

DNA microarrays exploit the ability of complementary strands of nucleic acids to base-pair with each other and bind (hybridise). Developers of the DNA microarray dot an array of DNA copies (cDNAs) corresponding to a large number of different mRNAs of known sequence onto a glass slide. In early microarray experiments, mRNA from one cell type was made into cDNA labeled with a red fluorescent dye, and mRNA from another cell type was made into cDNA labeled with a green fluorescent dye. The two cDNAs were then mixed, denatured and hybridized to the same DNA microarray. After washing away the unbound molecules, bound fluorescent nucleic acid samples were identified by laser microscopy. Fluorescent dots indicated expressed genes, and differences in microarray patterns between normal and cancerous cells could be quickly identified for comparative gene expression during diagnosis and treatment.

QUESTION 2: Yeast Biotechnology [10]

2.1 Briefly discuss how a yeast cell may be engineered into (i) producing useful plant secondary metabolites and (ii) “humanizing” (authentic glycosylation) proteins. (7)

Students choice of hijacking central metabolism Taxol or Artemisinic acid, and glycosylation of *P. pastoris*



Glycosylation:

- Knockout of *OCH1* (mannose transferring enzyme)
- Transporter genes isolated, transformed & targeted into Golgi membrane
- N-term localization signals fused in frame with mannosidae/transferase
- Expression of human genes that modify sugars in CMP-sialic acid

2.2 Briefly discuss the application of each of the following techniques:

2.2.1 TAR (1)

Isolation of specific chromosomal sections with the need to generate an entire library.

2.2.2 Yeast-two hybrid system (2)

Transcription factor DBD and AD fused to two different proteins for studying protein-protein interactions

QUESTION 3: Plant Biotechnology [10]

What are the genetic engineering strategies to create the following traits in transgenic crops. Where applicable, give an example of each:

a) Herbicide tolerance (5)

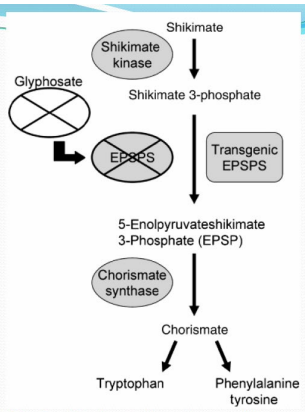
b) Virus resistance (5)

Expression of herbicide insensitive gene; degradation of herbicide or overexpression of herbicide target gene product.

Ex. Glyphosate – Roundup Ready & Touchdown, etc

Shikimate pathway links metabolism of carbohydrates to biosynthesis of aromatic compounds. In a sequence of 7 metabolic steps, phosphoenolpyruvate and erythrose 4-phosphate are converted to chorismate, the precursor of the aromatic amino acids and many aromatic secondary metabolites.

The shikimate pathway is found only in microorganisms and plants, never in animals.



Resistance to glyphosate in **RoundUp Ready** plants is engineered by expressing a form of the **5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EPSPS)** enzyme that is resistant to the herbicide.

b) Virus resistance.

- Exploitation of **pathogen-derived resistance (PDR).**
- Initial mild infection of virus confers protection against subsequent inoculation.
- **Host resistance strategies** have 2 mechanisms:
  - Protection by expression of native/modified viral proteins that interfere with viral replication cycle.
  - Protection at transcriptional level (gene silencing / sequence-specific RNA breakdown)

Transgenic resistance to Geminiviruses

(Shephard *et al*. 2009)

Contents lists available at ScienceDirect
Plant Science
journal homepage: www.elsevier.com/locate/plantsci
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Review

Transgenic strategies for developing crops resistant to geminiviruses

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- PDR (pathogen derived resistance) based approaches:
  - Coat protein mediated resistance (CPMR)
    - tomato plants expressing the CP of TYLCV exhibited delayed symptom development that was dependent on the expression levels of the transgenic CP (Kunik *et al.*, 1994)
  - Rep protein (viral replication and transcription) tolerance or strain specific immunity to ACMV, TYLCV-SV, ToLCNDV, BGMV, MSV - broader resistance.

Post-transcriptional gene silencing

- PTGS = RNA silencing or RNA interference (RNAi).
- Seq-specific breakdown mechanism in plants & eukaryotes which represents a natural antiviral defense mechanism.
- Involves cleavage of dsRNA to 21-25 nt siRNAs by Dicers.
- siRNAs interact with host proteins to form RNA-induced silencing complexes (RISC) - ds siRNAs are unwound and used as guides in the specific binding and destruction of targeted mRNA molecules.
- PTGS activated in transgenic plants by the introduction of dsRNAs homologous to viral sequences.
- Several studies reported RNAi resistance against geminiviruses:
  - ACMV -Vanderschuren *et al.*, 2009;

Non-PDR antiviral agents

- Mimicked the hypersensitive reaction (HR) used by plants at the initial site of infection.
  - Using the cell death-inducing **ribonuclease barnase** and **barstar** genes from *Bacillus amyloliquefaciens* (Taylor *et al.*, 2004; Pakniat *et al.*, 2010).
  - **Barnase** is a ribonuclease (**RNase**) and **barstar** its inhibitor. In the absence of geminivirus infection the 2 transgenes expressed at similar levels, *-no RNase production*. By placing barnase under the control of a viral virion-sense promoter that is activated during geminivirus infection, and barstar under the control of a viral complementary-sense promoter that is repressed during geminivirus infection, an infected cell should over-express barnase relative to barstar and die before the infecting virus can replicate and move.
  - Successfully applied for ACMV and *Tomato leaf curl virus-Australia* (TLCV-AU)
- Expression of DNA binding proteins (Rep-based artificial zinc finger proteins, Sera, 2005).
- Peptide aptamers that binds strongly to the Rep protein and interferes with its intracellular function.
- Use of **GroEL** homologues that bind to the viral CP as a tool to trap or capture viral particles and disrupt geminivirus infections or lessen their harmful effects (Akad *et al.*, 2007).

QUESTION 4: Molecular Diagnostics **[10]**

"HIV RNA (viral load) and CD4 T lymphocyte (CD4) cell count are the two surrogate markers of antiretroviral treatment (ART) responses and HIV disease progression that have been used for decades to manage and monitor HIV infection. Viral load is a marker of response to ART. A patient's pre-ART viral load level and the magnitude of viral load decline after initiation of ART provide prognostic information about the probability of disease progression. The key goal of ART is to achieve and maintain durable viral suppression. Thus, the most important use of the viral load is to monitor the effectiveness of therapy **after** initiation of ART."

4.1. Design an experiment in which you measure the viral load of HIV in blood plasma of patients that have received ART treatment. (7)

- Students need to suggest using RT-qPCR for absolute quantification of HIV in ARV-treated vs. pre-ART treated (same patient)
- Extract RNA from blood plasma.
- Treat RNA with DNAse and convert to cDNA using reverse transcriptase (since HIV is an RNA virus).
- Use primers design to target any of the HIV genes (eg envelop, pol, etc)
- Set up a standard curve of cDNA using 5 serial dilutions of known concentration
- Quantify the unknown viral load in ARV-treated and pre-ARV-treated samples by extrapolating from the standard curve.
- Calculate the difference therefore determine "the magnitude of viral load decline after initiation of ART"

4.2. "The co-circulation of different HIV types and groups can lead to dual infections and recombinants, which hinder diagnosis and therapeutic management". Suggest and justify ways in which you would modify your experiment approach in 4.1 in order to detect and measure different HIV types and groups HIV in ARV-treated vs. pre-ART treated patients. (3)

- SYBR green is used to detect dsDNA and therefore cannot discriminate between different HIV strains even if different primer sets will be used target each HIV type or group
- Change strategy to using Multiplex RT-qPCR instead using primer pairs targeting each HIV-type coupled with Taqman probes with multiples fluorophores attached for the simultaneous amplification and detection of more than one HIV target in a single reaction tube

QUESTION 5: Biotechnology-based Drug Discovery [10]

5.1. State the drug discovery process in sequence and indicate how long does this process take on average. (6)

Target Identification (or Selection); Target Validation; Assay (or Screen) development; Lead identification (or Hit to Lead); Lead optimization. This process takes 5-6 years on average.

5.2. What considerations should be made when selecting a suitable screening assay? (3)

This should be based on the specific target that is of interest. This would determine whether the assay is genomics, proteomics or metabolomics based. Considerations should also be made on the availability of equipment and access to infrastructure. Finally, the proposed assay should be amenable to high throughput screening.

5.3. What is the parameter during lead identification that measures the efficacy of the lead compound? (1)

The 50% Inhibitory concentration (IC_{50}) or effective concentration (EC_{50})

QUESTION 6: Vaccines [10]

List 4 differences between OPV and IPV polio vaccines, and the reason/s why we are slowly withdrawing OPV.

TYPES OF POLIO VACCINE

	ORAL POLIO VACCINE (OPV)	INACTIVATED POLIO VACCINE (IPV)
CONTAINS	Mixture of live, weakened poliovirus strains. *Trivalent OPV:* All three poliovirus types *Bivalent OPV:* Types 1 and 3 *Monovalent OPV:* Any one individual type	Mixture of inactivated, killed strains of all three poliovirus types.
HOW IT WORKS	Body produces antibodies in the blood and gut in response to the weakened virus. Helps stop transmission by limiting the virus's ability to replicate in the gut and spread to infect others.	Body produces antibodies in the blood in response to the inactivated virus. Protects the individual, but the virus may still replicate in the gut and can spread to infect others.
ADMINISTRATION	Easy, oral administration can be conducted by volunteers and is part of many countries' routine immunisation programmes. Used extensively in immunisation campaigns to root out poliovirus. Costs less than US$0.15 per dose.	Vaccine injection is administered primarily through routine immunisation programmes by trained health workers. Per-dose cost starting at US$1 for low-income countries, through Gavi mechanisms.
USE	Extremely effective in protecting children from WPV and cVDPV. Nearly every country has used OPV to stop wild poliovirus transmission because it prevents person-to-person spread of the virus, protecting both the individual and the community.	Extremely effective in protecting children from polio disease due to WPV and cVDPV, but cannot stop spread of virus in a community.
CVDPV RISK	On very rare occasions, in areas with under-immunised populations, the live weakened virus originally contained in OPV can mutate and spread, causing cVDPV.	Cannot cause cVDPV.
VAPP RISK	There are 3-4 vaccine-associated paralytic polio (VAPP) cases per million births. There are no outbreaks associated with VAPP.	Cannot cause VAPP.

OPV has the potential to mutate and cause disease
For eradication of wild poliovirus (the end game strategy), it would no longer be justified to use OPV, risks outweigh benefits

QUESTION 7: Stem Cells [10]

7.1 In stem cell-based therapies, the cells that are used within the therapy can be sourced in two different methods, either autologous or allogeneic. What is meant by these two terms? (2)

Hematopoietic stem cell transplantation (HSCT) is the new name for bone marrow transplantation. ... **Stem cell** transplantation refers to a process whereby the patient's HSCs are replaced by new **cells** (**either** from yourself [**autologous**] or someone else [**allogeneic**] that grow into a healthy **hematopoietic**system.

7.2 CAR-T therapy is a combination of genome engineering and cellular replacement therapy that is at the forefront of precision medicine. Discuss the concept behind the therapy including advantages and disadvantages of the treatment. (8)

Chimeric antigen receptor T cells: Engineering a patient's own immune cells (T-Cells) or a healthy donar T-cells to attack tumor cells within a patient (adoptive cell transfer).

By genetic engineering of T-Cells (using CRISPR, TALENS etc), to produce specific receptors (CAR'S), it will allow them to recognize tumor cells. For example, the addition of the CD19 receptor, for the CD19 antigen found on all B-Cells, it can be used to target Leukemias.

The Car-T cells are then expanded in the lab, infused into the patient, where they recognize the cancer cells and kills them.

For UCART19, the therapy is T-Cell immunotherapy against CD19+ B cell leukemias.

The TCR alpha gene is knocked out, which eliminate TCR expression and stops graph versus host disease. The CD52 gene is also knocked out.

Used to target childhood Acute Myeloid Leukemia, e.g. Celectis in conjunction with scientists and clinicians at Great ormond Street in London a year ago.

Addition of CD19 which targets cancer cells (modifications for different types of cancer?)

can be used for autologous transplantation, however cell number an issue. Allogeneic would be better.

Therefore need to prevent GvHD to prevent recognition of foreign cells: possible methods, using engineered suicide genes or genome engineering, can remove HLA proteins on the

donor T cells themselves so they are not recognised by the body's immune system, or remove the protein on the T cells responsible for recognising foreign cells (in the recipient body) and so prevent the T cells from attacking the recipient-donor.

Difficulties in growing up numbers of T cells: future work could involve modifiying HSC populations and generating large numbers of T cells however this method is not yet optimised and HSC are difficult to control in a dish - can often lead to acquired mutations.

QUESTION 8: Biofuels **[10]**

8.1 Various feedstocks are used to produce biofuels. Using corn as a feedstock, explain in detail the two processes employed for corn processing.

(10 X ½ = 5)

Dry milling Dry milling simpler than wet milling
• Corn kernels milled with hammer mill or grinder
• Addition of water and heat to liquefy corn starch; thermostable α-amylase
Partially hydrolyzed starch maltodextrins & free Glc
• Cooling to 50-60ºC, pH 5; addition of glucoamylase
cleavage of single glucose units
• Critical parameter is maximizing Glc generation & minimizing reversion sugars
• Corn slurry-enzyme mix cooled to 40ºC & addition of S. cerevisiae
Fermentation over a few days; Glc to ethanol (9%).
While dry milling is less capital intensive, it also yields less ethanol per bushel of corn than wet milling.

Wet milling Steep corn with water & sulfur dioxide to swell corn
40-60 hrs with lactic-acid fermentation by *Lactobacillus*
• Separation of corn pericarp, starch, germ & soluble portion
• Series of separation processes to yield highly purified corn starch & corn oil
• Highly purified corn starch is largest product by weight
• Starch can be hydrolyzed (acid)
• Starch conversion at high T with α-amylase to high MW oligomers
• Maltodextrin coverted to >95% Glc, higher purity than dry mill process
• HFCS produced from xylose/glucose isomerase
Purified Glc is versatile & C source for many fermentative processes
Higher value products e.g. antibodies, amino acids, vitamins

8.2 Biotechnology is the exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms to produce desired products. Using any two genetic manipulation approaches, explain

how you will enhance both cellulose break down and sugar fermentation. Use 1 approach for cellulose and 1 approach for sugar fermentation (5)

6 biotechnological approaches are discussed in class (with reference articles) namely:
Directed evolution, DNA shuffling, Metabolic engineering, Classical mutation and selection or plant breeding, Recombinant DNA, System biology.
Students should use 1 approach for each process and explain how these can be using to enhance the processing.

QUESTION 9: Crystallography **[15]**

9.1 In your crystallography practical in July 2019, you attempted purification of two protein components that together were meant to crystallize.

9.1.1 The target protein MAGEB1 has a molecular weight of 29kDa, and the nanobody has 13k kDa. After purification, you have 80ul of MAGEB1 at a concentration of 5 mg/ml, and 130ul of the nanobody at 3 mg/ml. What volumes of each solution must you mix together to yield a solution where the nanobody is in 2x molar excess? Show your calculations. (2)

n(target) = (5/29 mM) * 80ul = 14 umol n(nanobody) = (3/13 mM) * 130ul = 30 umol
So add 2*14*130/30 = **121ul** of nanobody to all **80ul** of the target protein.

9.1.2 Explain the role of the Nickel in the IMAC purification. (1)

Chelates the histidines of the poly-histidine tail, thus binding the protein to the beads.

9.1.3 During purification, after adding the crude lysate to the IMAC column and doing several wash steps, you had to do a pre-elution with low concentrations of imidazole. Explain why this was necessary, and how the imidazole addresses this. (3)

Any proteins will stick to IMAC if they have multiple histidines on the surface close enough to chelate to the nickel ion (1). They can also stick non-specifically to the agarose of the beads themselves (1). The low imidazole displaces the weaker surface interactions, and increases ionic strength to reduce the non-specific interactions.

9.2 Apart from intrinsic properties of a protein, describe aspects of the experiment that are in your control that can improve chances of getting the protein to crystallize. (3)

Any three of these (3):

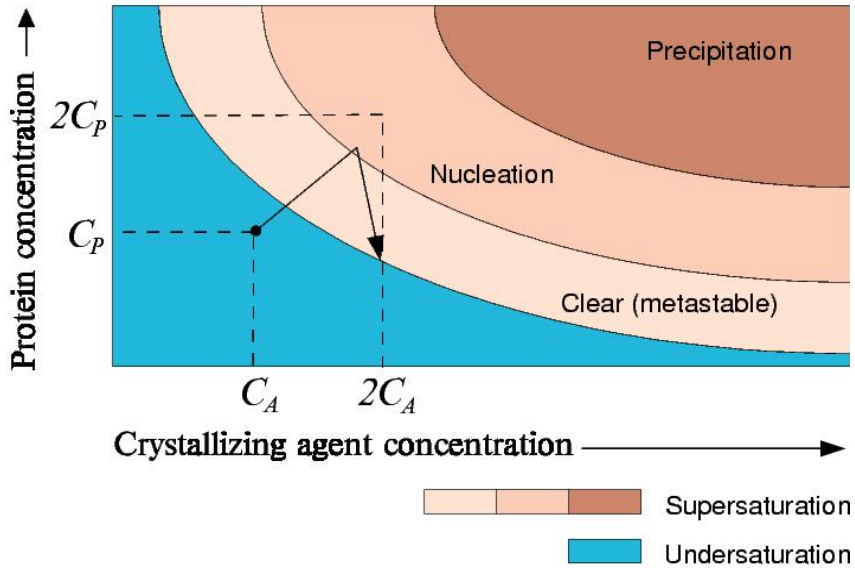
- More protein by improving expression – better media, larger flasks, larger media volume, better aeration, closer monitoring of cell density
- Improve gel quality for better assessment of purity
- More complete TEV cleavage – i.e. fresh TEV, longer cleavage time
- More IMAC resin, rerun if first time not great
- (Any other well-reasoned arguments)

9.3 Discuss how entropy drives both micelle formation and therefore protein folding. (3)

Proteins and micelles turn their hydrophobic parts inward, away from water. To solubilise hydrophobic groups, water must form ordered clathrates – low entropy state. Removing these groups from the water releases the water giving the molecules more degrees of freedom – a higher-entropy state.

9.4 Describe a general strategy for achieving larger crystals simply by changing concentrations of crystallization components, and explain the principle; sketch a schematic phase diagram if it simplifies your answer. (3)

Increase protein concentration, decrease precipitant concentration. It works because the system spends more time in the metastable zone. 3rd point for either describing the phase diagram correctly, or drawing something like this.



QUESTION 10: Application of knowledge in project **[5]**

During your individual BICX00 research project, you made use of "omics" (genomics, proteomic, metabolomics, *etc.*) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.

Pending of student choice. Aspects to address would be:

Sample type and isolation

Method of analysis

Control *versus* treated

Interpretation of results