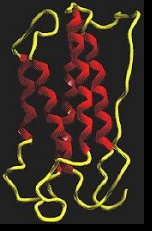

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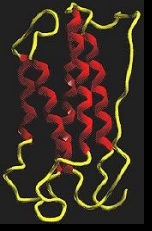
DESIGN PROJECT FOR

PRODUCTION OF IFN-ALPHA

IPRO 345

Chemical Engineering Design IPRO



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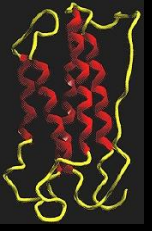
Objectives

- Design a process for production of the biotherapeutic compound IFN-Alpha from Chinese Hamster ovaries
- Assess whether the production design of this biotherapeutic compound is economically feasible and profitable



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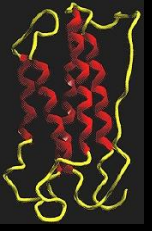
Background to Interferons

- Appear early after viral infection locally and systematically to limit spread of viral infection
- Inhibit viral activity by preventing RNA replication of the invading virus and certain other types of antigens and mark out tumor cells to be destroyed.
- Three naturally occurring forms: alpha, beta and gamma.



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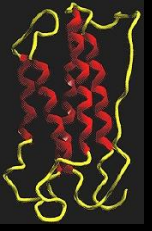
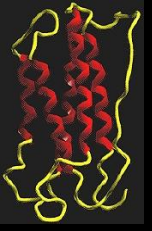
Background of Interferon-Alpha

- B-lymphocytes are the cellular producers of INF-alpha
- IFN-alpha is a multifunctional immunomodulatory cytokine
- IFN-alpha was approved by the Federal and Drug Administration (FDA) on February 25, 1991 to treat hepatitis C



Uses of Interferon-Alpha

- IFN-alpha remains the most frequently used IFN for both research and clinical applications
- Anti-viral applications such as chronic Hepatitis B and C now make up the bulk of IFN sales



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Outline of IFN-alpha production

Isolate IFN-alpha gene

↓

Ligate into Histag-vector

↓

Transfect into CHO cells

↓

Grow in batch reactor system

→

Elute IFN-alpha in Ni^{2+} affinity column

↑

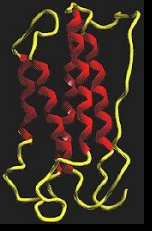
Centrifuge

↑

Ultrafiltration

↑

Freeze-drying



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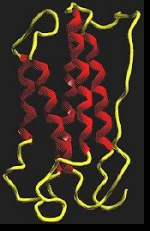
Cell Preparation

- Stimulation of T-cells by activator
- T-cells are collected and undergo RNA isolation
- Real Time RT-PCR to amplify the IFN-alpha gene
- PCR Amplification of IFN-alpha gene with restriction enzyme sites encoded on each end
- Cloning of the IFN-alpha insert into an appropriate cloning vector to verify correct PCR amplification



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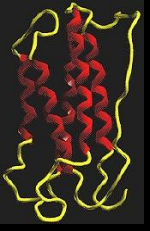
- IFN-alpha will be excised from the cloning vector and incorporated into the pSecTag2/Hygro vector to eventually allow for purification of Histidine-tagged proteins

- The IFN-alpha/pSecTag2/Hygro vector can be appropriately transfected into CHO cells to allow for the secretion of IFN-alpha

- A batch reactor system will be used for production



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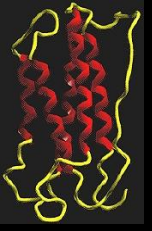
Histidine-tagged protein purification

- “His-tag” is the most commonly used tag for the facilitation of the purification of expressed recombinant proteins by affinity chromatography.
- A protein containing a histidine-tag is selectively seperated based on affinity to a metal-ion charged medium.



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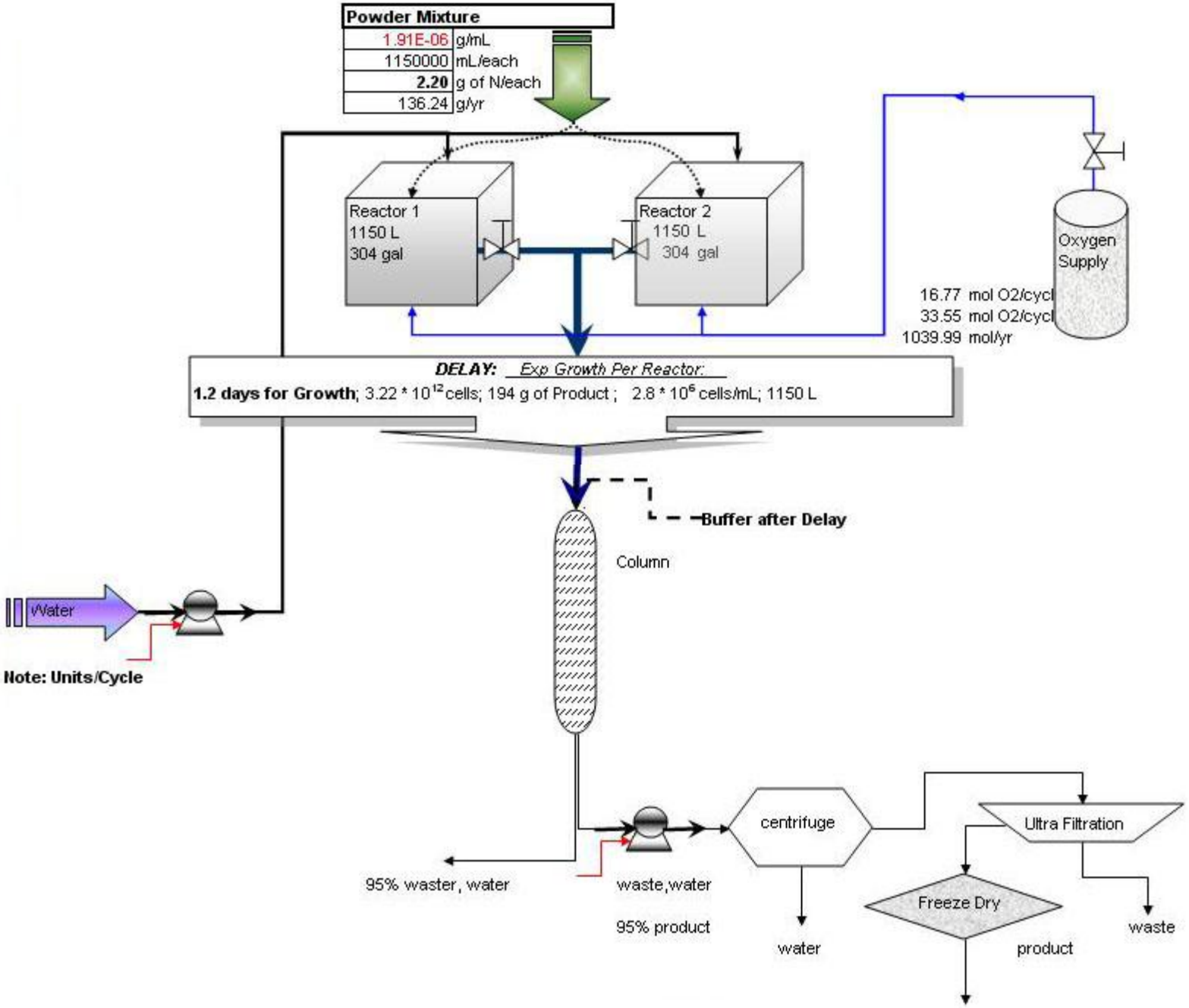
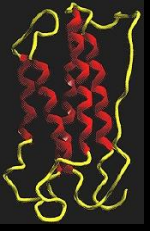
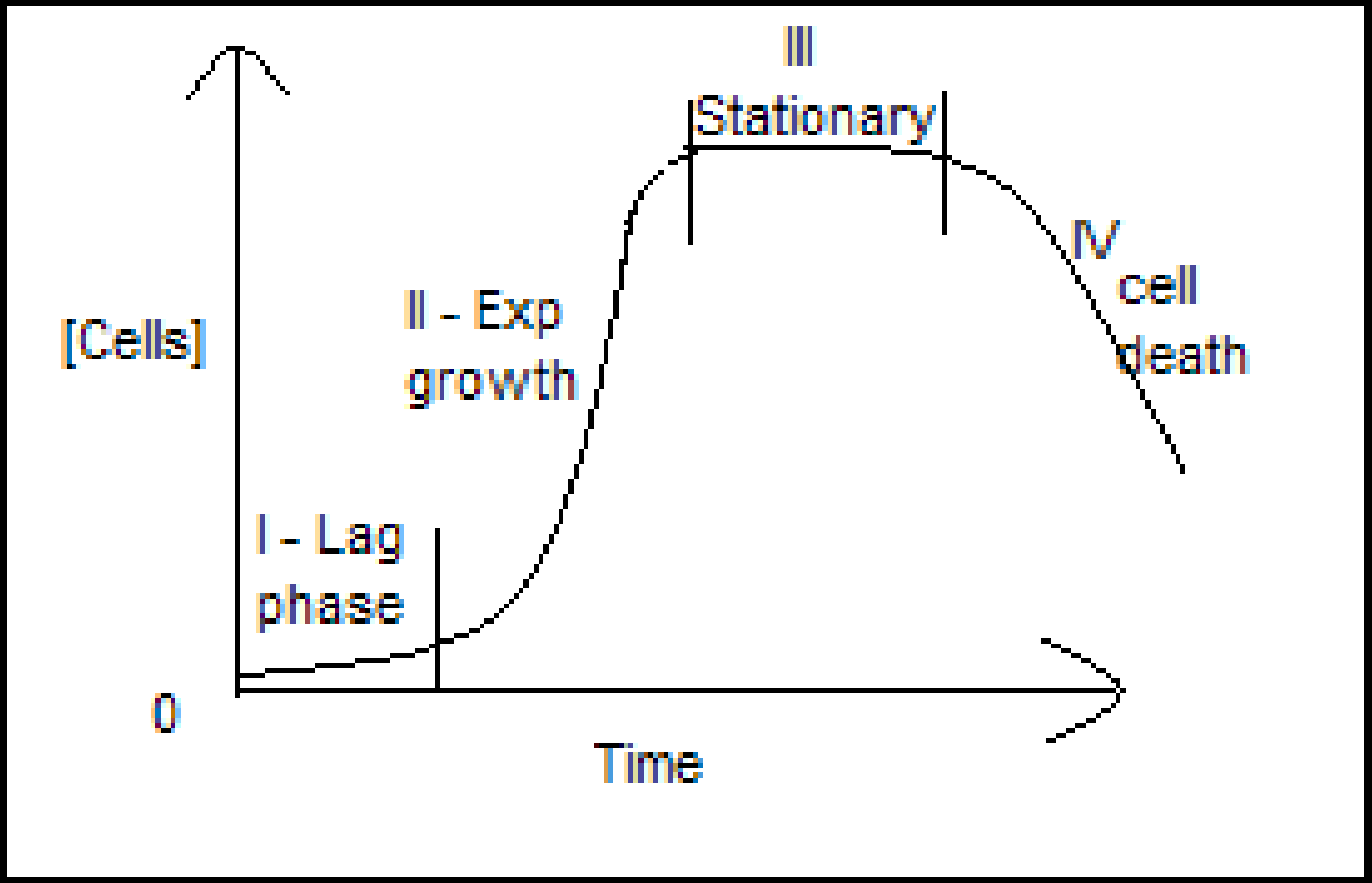
Nickel-affinity column

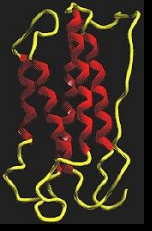
- A gravity flow purification system which allows for separation of histidine-tagged proteins.
- Histidine-tagged proteins will be bound to the resin based on their Ni^{2+} affinity once a binding buffer is passed through the column.
- An elution buffer can be used to separate only the successfully histidine-tagged IFN-alpha proteins.


Powder Mixture
1.91E-06 g/mL
1150000 mL/each
2.20 g of N/each
136.24 g/yr
Reactor 1
1150 L
304 gal
Reactor 2
1150 L
304 gal
Oxygen Supply
16.77 mol O2/cycl
33.55 mol O2/cycl
1039.99 mol/yr
DELAY: Exp Growth Per Reactor:
1.2 days for Growth; 3.22 * 10^12 cells; 194 g of Product ; 2.8 * 10^6 cells/mL; 1150 L
Buffer after Delay
Column
Water
Note: Units/Cycle
95% waster, water
waste,water
95% product
centrifuge
water
Ultra Filtration
Freeze Dry
waste
product



Cells + Nutrients → More cells + Products





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Batch Reactor Cell Growth Equations

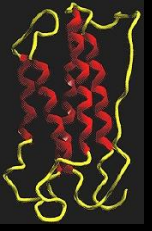
$$\frac{dC_c}{dt} = r_g - r_d \qquad C_c = C_{c0}(e^{\mu t}) \qquad C_c = C_{c0} e^{\left[\mu_{max}\left(\frac{C_s}{C_s + K_s}\right)t\right]}$$

Based on constraint of given variables and specified production output, two 1150 L batch reactors in parallel were designed

388 g of IFN-Alpha/cycle

30 cycles/year

Production Rate : 10 kg/yr



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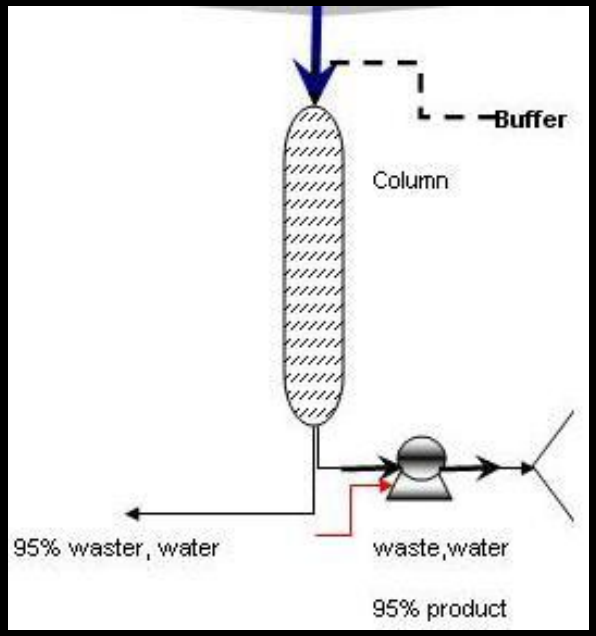
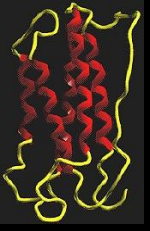
Separation

Ni-Affinity Absorption Column



Resin attaches to IFN-Alpha and after water is removed buffer is added for detachment



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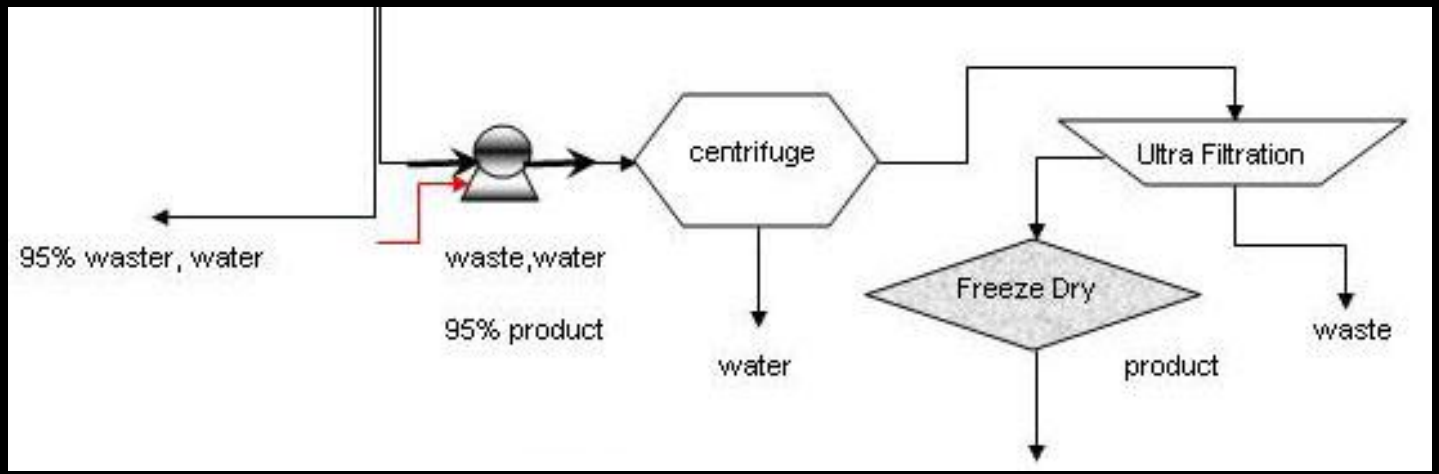
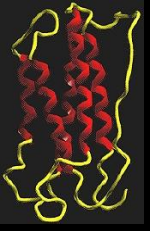
Separation Stages

Centrifuge: Uses rotation around fixed axis so centrifugal force is used for separating materials based on densities

Ultra filtration : Uses pressure through a semi-permeable membrane with pores sized to retain solids and pass water

Freeze Drying: Removes water from the food matrix by sublimation and is useful for sensitive and high-valued fluids





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GANTT CHART

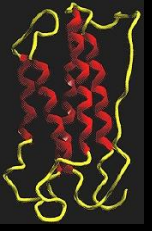
A chart that depicts progress in relation to time, often used in planning and tracking a project.

G1= Growth
C1=Clean
S1=Separation 1

Gantt Chart

days

	1	2	3	4	5	6	7	8	9	10
G1										
C1										
S1										



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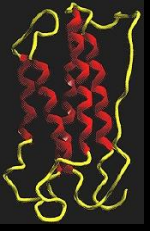
Cost Estimate

- Total bare module cost for fabricated equipment, CTFE: $186,000.
- Total direct permanent Investment CDPI: $416,600.
- Total Depreciable capital CTDC: $561,100.
- Total permanent investment: $639,700.



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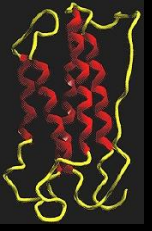
Approximate Profitability Analysis

- Minimum Proposed price of IFN-alpha: 1mg = 40 cents.
- Sales of 10kg of IFN-alpha: $ 4million.
- Total production cost: $ 3.5million.
- Pre-tax Earnings: $463,000.
- Net Earnings: $292,000.
- Process is Profitable.



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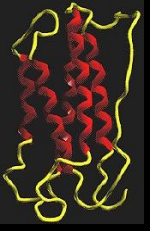
Approximate Profitability Analysis

- 30 % return on Investment.
- Payback Period: 1.667yr
- Process is still profitable at subsidized price.



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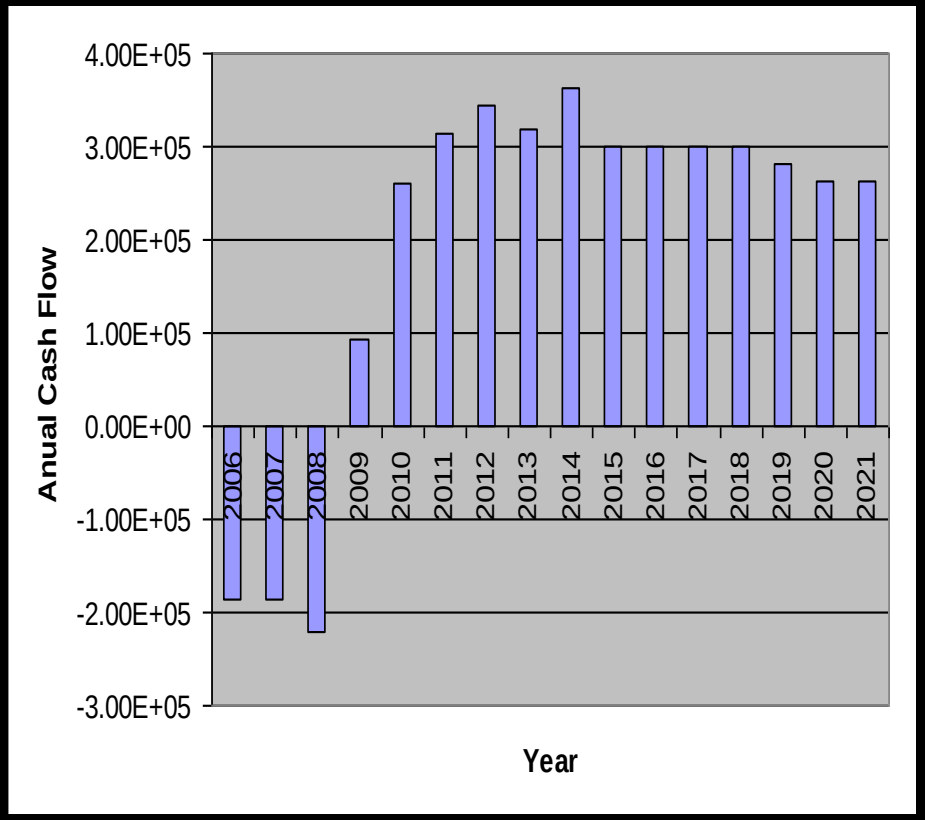
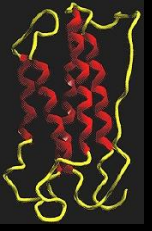
Rigorous Profitability Analysis

- Investor's return rate: 23.76%.
- Emphasizes profitability.



Discounted Annual Cash Flow Over 15 yrs



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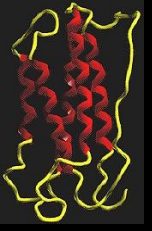
Humanitarian Consideration

- High Price of product is a deterrent to patients.
- A lot of people need this product.
- Give out certain amount of free products.
- Sell at subsidized price.
- Suggested Selling price: 1mg = 40 cents.



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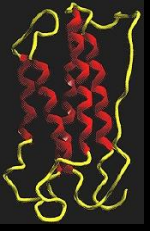
Conclusion

- Large scale production of IFN-alpha would increase supply to meet its demand.
- Positive effects on patients.
- More Competitive industry.
- Reduce high price of product.



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Questions

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