

Problem Set 4 - partial

(Due Sept 23rd)

1. As we discussed in class, there are several computational algorithms that allow biochemists to predict secondary structure. Let's see how good these algorithms hold up to real structural information.
 - a. Access the following two pdb files: 1DNK and 7ACN.
 - i. What are these proteins and what organism do they come from?
1DNK = DNase I from *Bos Taurus* 7ACN = Aconitase from *Sus scrofa* (pig)
 - ii. Exactly what position do they occupy in the genome (I showed you how to do this in class). Make sure to identify the chromosome and position.
1DNK – chromosome 25: 2,977,213 – 2,995,476
7ACN – chromosome 5: 4,385,951 – 4,443,595
 - b. Use the Chou-Fasman prediction program on ExPASy to predict the secondary structure. Compare this prediction with the actual secondary structure observed in the pdb file. You can do this visually using Chimera or by observing the text in the pdb file – either way, map the structure vs. prediction and determine how good the prediction is. **In general, there is reasonable agreement. The model gets some sections very wrong.**
 - c. Based on the observed secondary structure, predict what the CD spectrum of each protein might look like. Please justify your sketch. **DNase I is mostly β sheet, so I draw it looking mostly like an all β protein. Aconitase is about 2x more α helix than β sheet, so the spectrum is drawn to reflect primarily helix.**

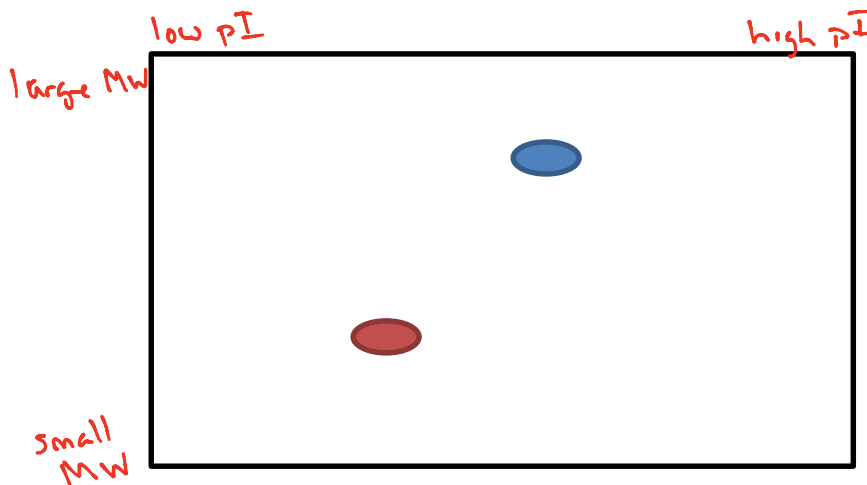


2. Using the tools in ExPASy, determine the pI, MW, and molar absorptivity of each protein.

1DNK pI = 5.08 MW = 29065.6 g/mol $\epsilon_{280} = 39100 \text{ M}^{-1}\text{cm}^{-1}$

7ACN pI = 7.20 MW = 82693.1 g/mol $\epsilon_{280} = 80050 \text{ M}^{-1}\text{cm}^{-1}$

3. Assuming that you start with a homogenous mixture of both of these proteins (and nothing else), predict what a 2D gel electrophoresis experiment would look like. 1ACN in Blue, 7DNK in red.



4. For 1DNK, predict the MW of all peptides produced when it is treated with Cyanogen Bromide (CNBr). You are encouraged to use ExPASy to determine MW values, but you should manually determine where the peptide chain will be broken.

LKIAAFNIRTFGETKM MW = 1840.2 g/mol
 SNATLASIYIVRIVRRYDIVLIQEVRDShLVAVGKLLDYLNQDDPNTYHYVVSSEPLGRNSYKERYLFLFRPNKVSVLDTYQY
 DDGCESCND SFSREP AVVKFSSHSTKVKEFAIVALHSAPSDAVAEINSLYDVYLDVQQKWHLNDVM MW = 16970
 g/mol
 LM MW = 244.4
 GDFNADCSYVTSSQWSSIRLRTSSTFQWLI PDSADTTATSTNCAYDRIVVAGSLLQSSVVP GSAAPFDFQAAYGLSNEM
 MW = 8436.2
 ALAISDHYPVEVTLT MW = 1628.8

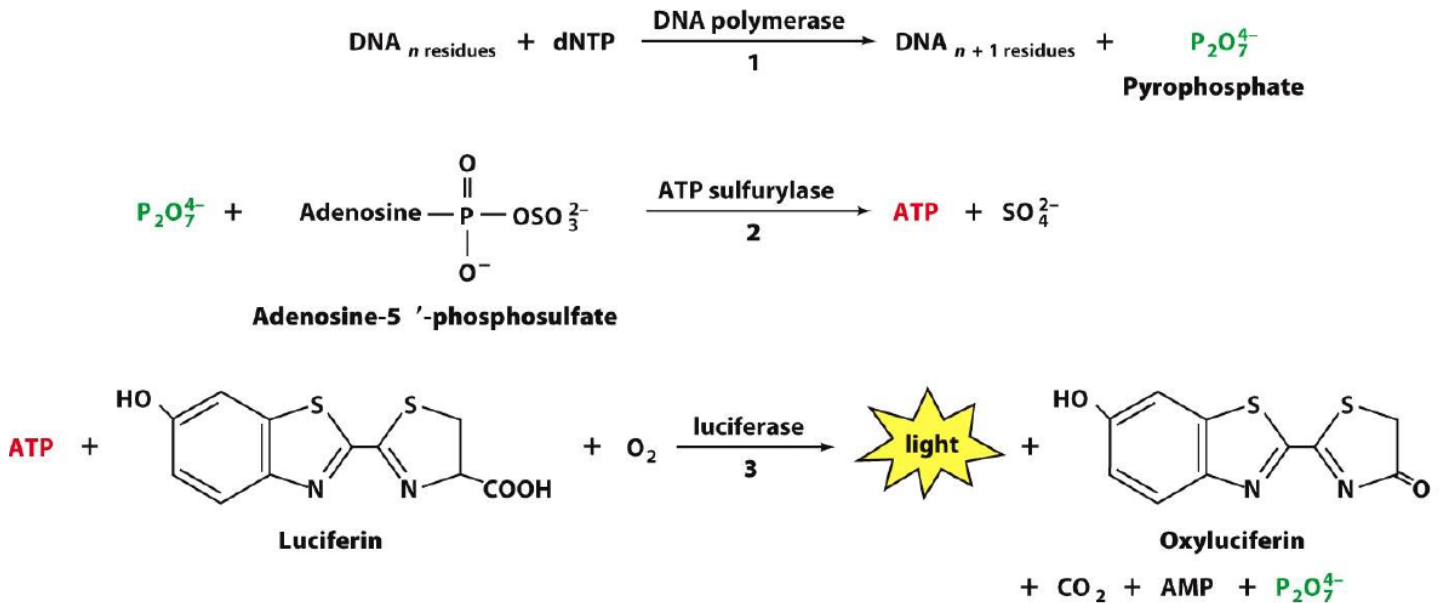
5. Use the PeptideCutter tool in ExPASy to predict what fragments would be produced when 7ACN is digested with each of the following proteases. In this tool, make sure to select “only the following selection of enzymes and chemical” option. Also, it is quite helpful to choose the “Table of sites” display option. **See attached**
- Chymotrypsin
 - Arg-C
 - Thrombin – **No cleavage sites**
6. A tandem MS experiment results in peaks at the following m/z ratios. Determine the sequence of this peptide. **GluAsn(Ile or Leu)TyrPheGlnGlyGln**

128.2	185.2	313.3	460.5	623.7	736.9	851	980.1
Gln	Gln+Gly	Gln+Gly+Gln	Q+G+Q+F	...F+Y	...Y+L or I	...L/I + N	...N+E

A peak is also observed at m/z = 425.5. What is the source of this peak? **This is the +2 peak of the 851 Da peptide. Since it carries a charge of +2, it will be observed at ½ the mass.**

7. Using the attached electropherogram:
- Please describe how this data is generated. **A PCR reaction is carried out with a small fraction of 2'3'-didexonucleic acids. Each base on the ddNTP is modified with a chromophore. When the modified ddNTP is incorporated into the growing DNA chain, the elongation is terminated. This terminating base is modified with the complementary base, so you know exactly what base is at a given position. These PCR fragments are separated by capillary electrophoresis and the resulting electropherogram provides single nucleotide separation so the absorbance vs. time profile can be used to determine the sequence.**
 - Determine the sequence of nucleotides 50-100 (feel free to simply highlight the sequence on the image). **See attached**
 - Discuss why there are regions that are not useful and highlight those regions. **This represents oligonucleotides that are too short to accurately separate by electrophoresis and/or single ddNTPs that absorb strongly.**

8. What is meant by pyrosequencing? What reactions are important in this process? Pyrosequencing refers to the dependence on pyrophosphate production during the sequencing reaction. A dNTP is passed across the sequencing plate. If the complementary base is present on the next position for elongation, the coupling reaction will happen and pyrophosphate will be produced. This pyrophosphate will react with a modified AMP (sulfate on the alpha phosphate) to produce ATP. The ATP then reacts with luciferin, catalyzed by luciferase, to produce a burst of light via chemiluminescence. The slide is washed and another dNTP is added.



9. A protein is independently digested with Arg-C and Asp-N. Identify this protein.

Asp-N	Arg-C
DSG	EIVR
DLT	LDLAGR
DIRK X	AVFPSIVGR
DSYVG	GYSFVTTAER
DLAGR	DLTDYLMKILTER
DETTALVC	VAPEEHPTLLTEAPLNPKANR
DEAGPSIVHR	KDLYANNVMSGGTTMYPGIADR
DIKEKLCYVAL X	HQGVMMVGMGQKDSYVGDQAQSKR X
DNGSGLVKAGFAG	MQKEITALAPSTMKIKIAPPER
DLYANNVMSGGTTMYPGIA	DEDETTALVCDNGSGLVKAGFAGDDAPR X
DGVTHNVPIYEGYALPHAIMRL	TTGIVLDSGDGVTHNVPIYEGYALPHAIMR
DFENEMATAASSSLEKSYELP X	EKMTQIMFETFNVPAMYVAIQAVLSLYASGR

DEAQSQRGILTLKYPIEHGIITNW X	GILTLKYPIEHGIITNWDDMEKIWHHTFYNELR
DYLMKILTERGYSFVTTAEREIVR	CPETLFQPSFIGMESAGIHETTYNSIMKCDIDIR X
DAPRAVFPSIVGRPRHQGVMMVGMGQK X	KYSVWIGGSILASLSTFQQMWITKQEYDEAGPSIVHR
DGQVITIGNERFRCPETLFQPSFIGMESAGIHETTYNSIMKC X	DIKEKLCYVALDFENEMATAASSSSLEKSYELPDGQVITIGNER X
DRMQKEITALAPSTMKIKIIPPERKYSVWIGGSILASLSTFQQMWITKQEY	
DMEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKANREKMTQIMFETFNPAMYVAIQAV X	
LSLYASGRRTTGIVL	

Blasting this sequence tells you that the protein is **Actin**.

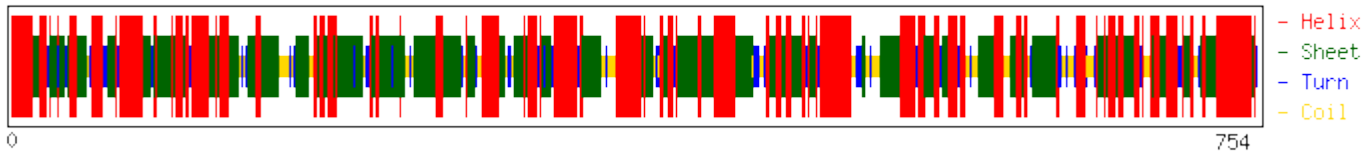
DEDETTALVCDNGSGLVKAGFAGDDAPRAVFPSIVGRPRHQGVMMVGMGQKDSYVGD
 DEAQSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKAN
 REKMTQIMFETFNPAMYVAIQAV

Name of the sequence is 7ACN.

Sequence consists of 754 amino acids.

Target Sequence:

```
ERAKVAMSHF EPHEYIRYDL LEKNIDIVRK RLNRPLTLSE KIVYGHLDLP ANQEIERGKT YLRLRPDRVA
MQDATAQMAM LQFISSGLPK VAVPSTIHCD HLEAQLGGE KDLRRAKDIN QEVYNFLATA GAKYGVGFWR
PGSGIIHQII LENYAYPGVL LIGTDSHTPN GGGLGGICIG VGGADAVDM AGIPWELKCP KVIGVKLTGS
LSGWTSPKDV ILKVAGILTV KGGTGAIVEY HPGVDSISIC TGMATICNMG AEIGATTSVF PYNHRMCKYL
SKTGRADIAN LADEFKDLHLV PDPGCHYDQV IEINLSELKP HINGPFTPD L AHPVAEVGSV AEKEGWPLDI
RVGLIGSCTN SSYEDMGRSA AVAKQALAHG LKCKSQFTIT PGSEQIRATI ERDGYAQLR DVGIVLANA
CGPCIGQWDR KDIKKGEKNT IVTSYRNFT GRNDANPETH AFVTSPEIVT ALAIAAGTLKF NPETDFLTGK
DGKFKLEAP DADELPRAEF DPGQDTYQHP PKDSSGORVD VSPTSQRLQL LEPFDKWDGK DLEDLQILIK
VKGKCTTDHI SAAGPWLKFR GHLDNISNNL LIGAINIENR KANSVRNAV T QEFGVPD TA RYYKQHGIRW
VVGIDENYGE GSSREHSALE PRHLGGRAII TKSFARIHET NLKKQGLLPL TFADPADYNK IHPVDKLTIQ
GLKDFAPGKP LKCIKHPNG TQETILLNHT FNETQIEWFR AGSALNRMKE LQQK
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Secondary Structure:

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Query 1      *      *      *      *      *
ERAKVAMSHFEPHEYIRYDLLEKNIDIVRKRLNRPLTLSEKIVYGHLDLDPANQEIERGKT 60
Helix 1      HHHHHHHHHHHHHHH HHHHHHHHHHHHHHH HHHHHHH HHHHHHHHH 60
Sheet 1      EEEE      EEEEE      EEEEEEEEEEEEEEEEEEEEEEE E 60
Turns 1      T      T      T      T      T      T      T      T      TTT 60

Query 61     *      *      *      *      *
YLRLRPDRVAMQDATAQMAMLQFISSGLPKVAVPSTIHCDHLEAQLGGEKDLRRAKDIN 120
Helix 61     HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH HHHHHHHHHHHHHHHHHHHHH 120
Sheet 61     EEE      EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE EE 120
Turns 61     TT      T      T      T      T      T      T      T 120

Query 121    *      *      *      *      *
QEVYNFLATAGAKYGVGFWRPGSGIIHQIILENYAYPGVLLIGTDSHTPNGGGLGGICIG 180
Helix 121    HHHHHHHHHHHHHHH HHHHHHHHHHH HH 180
Sheet 121    EEEEEEE      EEEEE      EEEEEEEEEEEEEEEEEEEEEEE EEEEEEE 180
Turns 121    T      T      T      T      T      T      T      T 180

Query 181    *      *      *      *      *
VGGADAVDMAGIPWELKCPKVIGVKLTGSLSGWTSPKDVILKVAGILTVKGGTGAIVEY 240
Helix 181    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH HHHHHHHHHHHHH HH 240
Sheet 181    E      EEEEEEEEEEEEEEEEEEEEEEEEEEE EEEEEEEEEEEEEEEEEEE 240
Turns 181    T      T      T      T      T      T      T      T 240

Query 241    *      *      *      *      *
HGPVDSISICTGMATICNMGAEIGATTSVFYPYNHRMCKYLSKTGRADIANLADEFKDLHLV 300
Helix 241    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH HHHHHHH HHHHHHHHHHHHH 300
Sheet 241    EEEEEEEEEEEEEEE EEEEEEEEEEE EEEEEEE EEEEEEE EEEEE 300
Turns 241    TT      T      T      T      T      T      T      T 300

Query 301    *      *      *      *      *
PDPGCHYDQVIEINLSELKPHINGPFTPDLAHPVAEVGSVAEKEGWPLDIRVGLIGSCTN 360
Helix 301    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH 360
Sheet 301    EEEEEEEEEE EEEEEEE EEEEEEE EEEEEEE EEEEEEE 360
Turns 301    TT      T      T      T      T      T      T      T 360

Query 361    *      *      *      *      *
SSYEDMGRSA AVAKQALAHGLKCKSQFTITPGSEQIRATI ERDGYAQLR DVGIVLANA 420
Helix 361    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH 420
Sheet 361    EEEEE EEEEEEEEEEE EEEEE EEEEEEEEEEEEEEEEEEE 420
Turns 361    T      T      T      T      TT      T      TT      T 420

Query 421    *      *      *      *      *
CGPCIGQWDRKDIKKGEKNTIVTSYRNFTGRNDANPETHAFVTSPEIVTALAIAAGTLKF 480
Helix 421    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH 480
Sheet 421    EEEEEEE EEEEEEEEEEE EEEEEEEEEEEEEEEEEEEEEEE 480
Turns 421    TT      T      TT      T      TTT      T      T      T 480

*      *      *      *      *
*      *      *      *      *
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Turns	121		T		T		T		T	180
		*		*		*		*		
Query	181	WSSIRLRTSSTFQWLIPDSADTTATSTNCAY	DRIVV	GSL	LQSSV	VP	GSAAP	FD	FQAAYG	240
Helix	181	HHHHHHHHHHHHHHHHHHHHHHHHHHHH		HHHHHHHHHHHHHH			HHHHHHHHHHHH			240
Sheet	181	EEEEEEEEEEEE		EEEEEEEEEEEEEEEEEEEEEEEEEEEE						240
Turns	181		T		T		T		T	240
		*		*						
Query	241	LSNEMALAI	SDHYF	VEV	TT					260
Helix	241	HHHHHHHHHHH		HH						260
Sheet	241		EEEEEE							260
Turns	241		T		T					260

ERAKVAMSHF	1175.371
EPHEY	673.68
IRY	450.538
DLLEKNIDIVRKRLNRPLTLSEKIVY	3139.732
GHLDDPANQEIERGKTY	1943.06
LRLRPDRVAMQDATAQMAMLQF	2563.049
ISSGLPKVAVPSTIHCDHLIEAQLGGEKDLRRAKDINQEVY	4502.125
NF	279.296
LATAGAKY	793.918
GVGF	378.428
W	204.228
RPGSGIIHQIILENY	1709.966
AYPGVLLIGTDSHTPNGGGLGGICIGVGGADAVDVMAGIPW	3879.417
ELKCPKIVIGVCLTGSLSGW	2015.442
TSPKDVILKVAGILTVKGGTGAIVEY	2630.12
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NHRMKKY	976.164
LSKTGRADIANLADEF	1720.9
KDHLVPDPGCHY	1380.541
DQVIEINLSELKPHINGPF	2163.458
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TITPGSEQIRATIERDGY	2007.188
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NRNF	549.587
TGRNDANPETHAF	1429.469
VTSPEIVTALAIAGTLKF	1831.183
NPETDF	721.722
LTGKDGKKF	993.171
KLEAPDADELPRAEF	1700.866
DPGQDTY	794.773
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DKW	447.491
DGKDLEDLQILIKVKGKCTTDHISAAGPW	3152.613
LKF	406.525
RGHLDNISNLLIGAINIENRKANSVRNAVQTQEF	3792.229
GPVPDTARY	975.069
Y	181.191
KQHGIW	924.073
VVIGDENY	907.976
GEGSSREHSALEPRHLGGRAITKSF	2793.095
ARIHETNLKKQGLLPLTF	2079.473
ADPADY	650.643
NKIHPVDKLTIQGLKDF	1966.311
APGKPLKCIKHPNGTQETILLNHTF	2871.393
NETQIEW	918.958
F	165.192
RAGSALNRMKELQK	1730.018

Chymotrypsin

ER	303.318
AKVAMSHFEPHEYIR	1815.08
YDLLEKNIDIVR	1490.72
KR	302.377
LNR	401.466
PLTLSEKIVYGHLDDPANQEIER	2637.929
GKTYLR	736.869
LR	287.362
PDR	386.408
VAMQDATAQMAMLQFISSGLPKVAVPSTIHCDHLIEAQ LGGEKDLR	4922.726
R	174.203
AKDINQEVYNFLATAGAKYGVGFWR	2819.172
PGSGIIHQIILENYAYPGVLLIGTDSHTPNGGGLGGICIG VGGADAVDMAGIPWELKC	13602.736
PKVIGVKLTGSLSGWTSPKDVILKVAGILTVKGGTGAV EYHGPGVDSISCTGMATICNM	
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MKKYLSKTGR	1211.488
ADIANLADEFKDLVDPGCHYDQVIEINLSELKPHING PFTPDLAHPVAEVGSVAEKE	7255.101
GWPLDIR	
VGLIGSCTNSSYEDMGR	1788.965
SAAVAKQALAHGLKCKSQFTITPGSEQIR	3041.519
ATIER	588.662
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NFTGR	593.64
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GHLDNISNLLIGAINIENR	2190.444
KANSVR	673.77
NAVTFEFGPVPDTAR	1601.736
YYKQHGIR	1064.212
WVIGDENYGEGSSR	1667.752
EHSALPR	938.008
HLGGR	538.607
AIITKSFAR	1006.213
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LLNHTFNETQIEWFR	
AGSALNR	687.754
MKELQQK	904.092

Arg-C

