Cellular Libraries of Peptide Substrates (CLiPS)

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Major: Biochemistry

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Important Roles of Proteases

- Proteases are enzymes that cleave proteins.
- Proteases help cancer cells to transfer from one place to another, and inhibiting proteases might prevent cancer cells from spreading out.

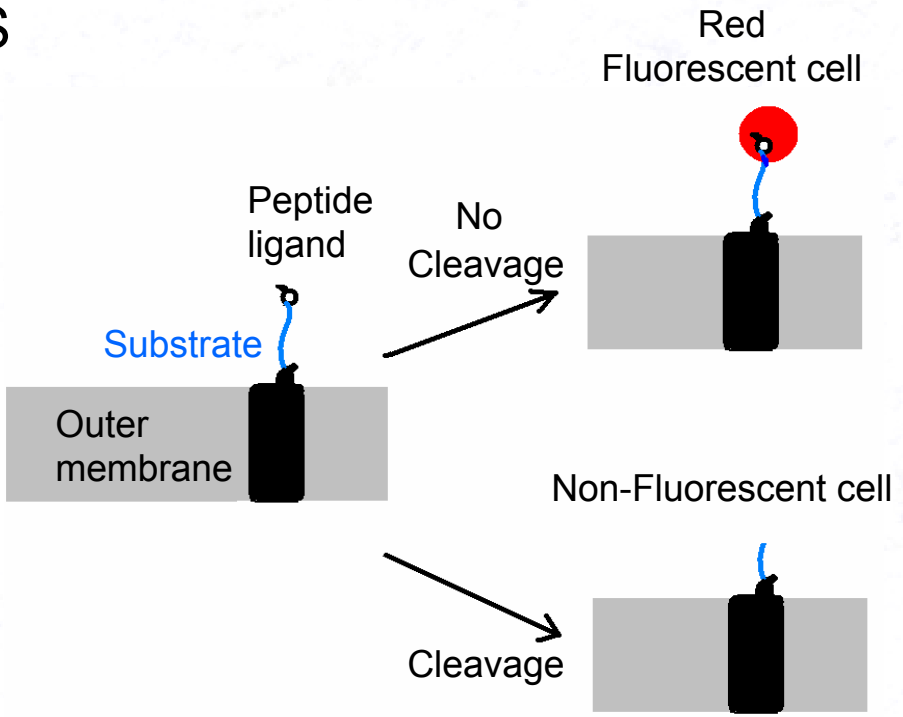
Example: Matrix metalloprotease-1 (MMP-1) degrades collagens which is part of extra cellular matrix (ECM). This degradation of ECM allows cell growth.

Research Goal:

Determine the optimum peptide sequences that protease can cleave.

Cellular Libraries of Peptide Substrates (CLiPS)

- Experimental Method: CLiPS
- Substrates are labeled with red fluorescent probe and peptide ligand bind to probe
- Red fluorescent cells are treated with protease
- Red fl. cell - No cleavage
  Non-fl. Cell - Cleavage
- Utilize Fluorescence Activated Cell Sorter (FACS) to detect substrate cleavage



What I do in the laboratory – Optimize Method

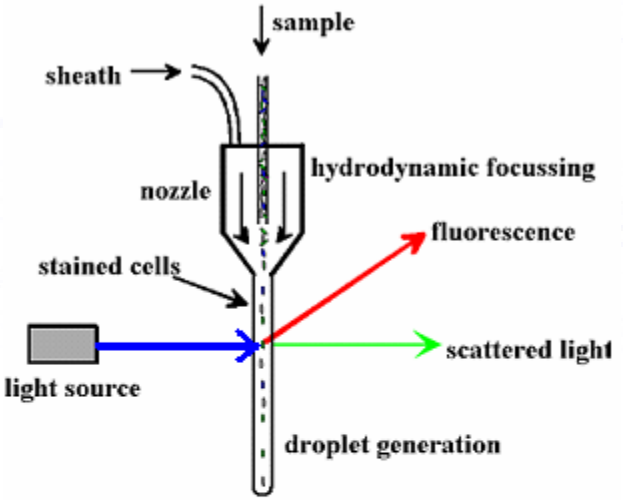
- Culture
- Subculture – control cell growth rate
- Induction – produce substrates
- Reaction – incubate cells with protease
- Label – label cells with red fluorescent probe
- Wash – remove unbound fluorescent probe from cells
- Run samples on FACS

An extra step towards better results that we found:

- Remove growth media from cells completely

Fluorescence Activated Cell Sorter (FACS)

BD FACSAria TM cell sorter – Flow Cytometer

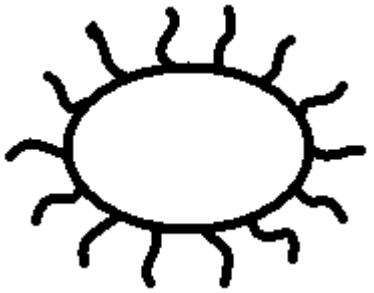


Fluidics System in FACS

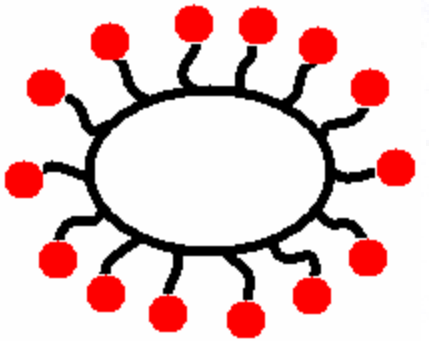
- Sheath center the sample stream to obtain an individual cell flows.
- As each individual cell flows through blue laser, lights are emitted from excited cell.

FACS

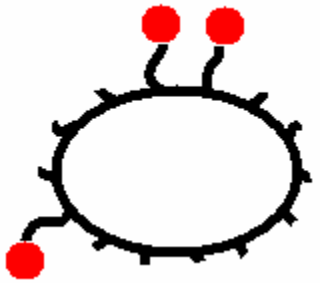
Expected Cell Population Analysis



Auto-fluorescence of cell



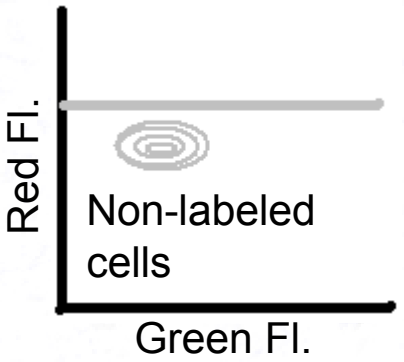
Uncleaved, labeled cell

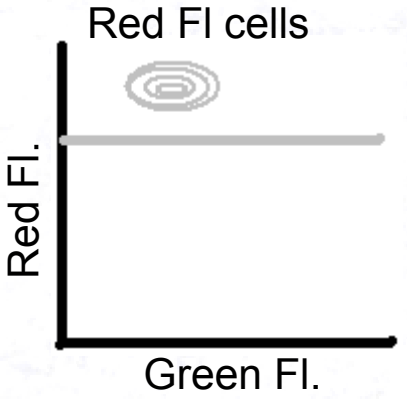


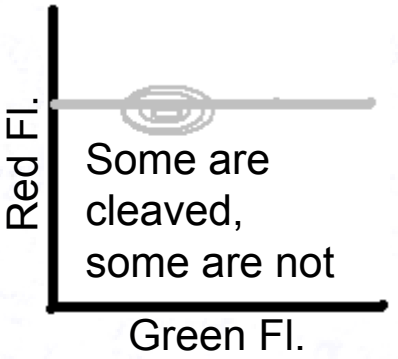
Cleaved, labeled cell

Red probe

Protease





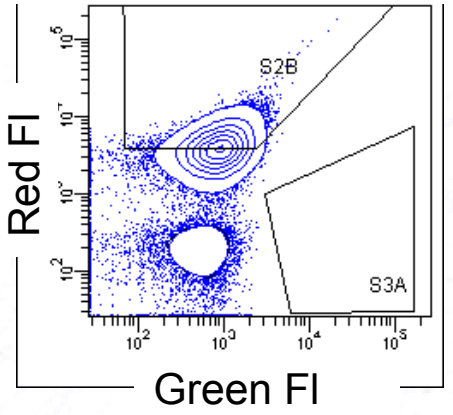


Key: Substrate Red fluorescent probe

Results

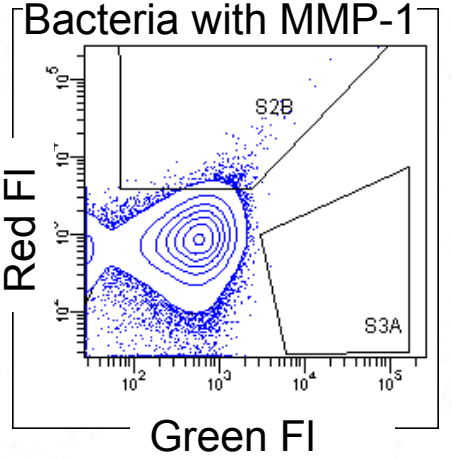
A.

Bacteria without MMP-1



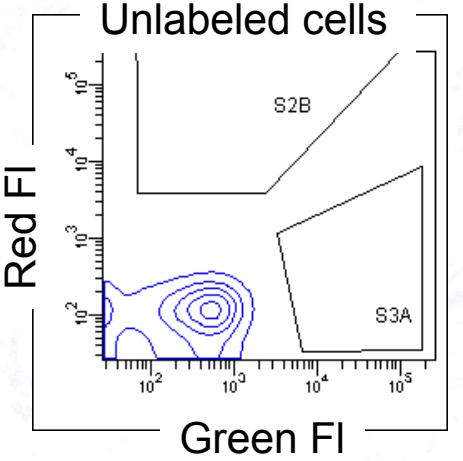
B.

Bacteria with MMP-1



C.

Unlabeled cells



Calculation of Conversion:

(Without MMP-1 cell mean A. *– With MMP-1 cell mean* B. *) /*
(Without MMP-1 cell mean A. *– auto-fluorescence of cells* C. *)*

E.g.
(3535 – 1065)/(3535 – 200) = 0.741
0.741 represents the average of 74.1% of the cell population cleave

Key : Each dot stands for one cell.

Peptide Sequences of MMP-1 Substrates

Cleave

Substrate	Sequence						Conversion%	Std Dev
	P4	P3	P2	P1	P1'	P2'		
G1	-	P	V	A	M	R	97	0.78
G2	-	P	V	N	V	V	96	4.77
F6	V	P	M	V	V	-	95	1.92
F2	T	P	L	A	L	-	94	0.95
D2	V	P	V	N	M	-	93	19.78
D4	M	P	L	V	M	-	93	3.39
H5	V	P	L	N	M	-	93	6.15
E3	-	P	V	P	M	V	88	2.66
A1	-	P	M	A	V	T	79	23.60
B2	V	P	V	V	M	-	78	5.62
E6	-	P	M	A	V	I	75	10.94
D5	M	P	V	V	L	-	70	3.39
Consensus	V	P	V		M			

Amino acid Abbreviation

L - Leucine
M - Methionine
P - Proline
V - Valine

Summary

- A new method of studying proteases – CLiPS
- Remove all the growth media off cells before labeling
- Run samples on FACS and analyze FACS data
- The optimum peptide sequence of MMP-1

Future Plan

- The high conversion samples we found this summer will be studied further

Acknowledgement

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- The other intern: David Lee
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- Centers of Cancer Nanotechnology Excellence (CCNEs)

Laboratory Group Members:

Group members are:(L-R) Professor Patrick Daugherty, Jerry Thomas, Claudia Gottstein, Annalee Nguyen, Laura-Marie Nucho, Karen Dane, Xia You, Marco Mena, Sejal Hall, Kevin Boulware, Sophia Kenrick, Yimin Zhu, and Jeffrey Rice.
Not pictured: Paul Bessette, Abeer Jabaiah.

Amino Acids Abbreviation

Abbreviation		Amino acid name
Ala	A	Alanine
Arg	R	Arginine
Asn	N	Asparagine
Asp	D	Aspartic acid (Aspartate)
Cys	C	Cysteine
Gln	Q	Glutamine
Glu	E	Glutamic acid (Glutamate)
Gly	G	Glycine
His	H	Histidine
Ile	I	Isoleucine
Leu	L	Leucine
Lys	K	Lysine
Met	M	Methionine
Phe	F	Phenylalanine
Pro	P	Proline
Ser	S	Serine
Thr	T	Threonine
Trp	W	Tryptophan
Tyr	Y	Tyrosine
Val	V	Valine

Plot