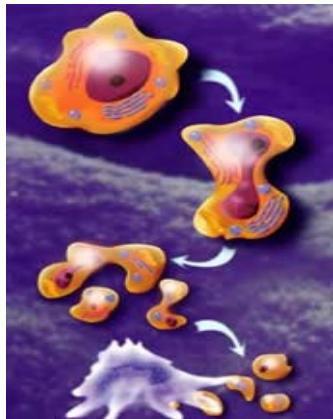




Structural Basis for Substrate Specificity of Executioner Caspases

Guoxing Fu
Dr. Irene Weber's lab
Department of Biology
Georgia State University



Apoptosis: Programmed Cell Death

Excessive apoptosis :

Stroke

Neuronal crush injury

Neurodegenerative diseases

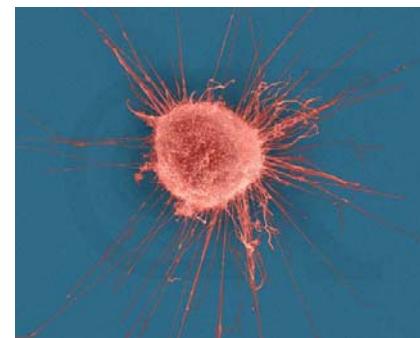


Normal Brain & Brain with Alzheimer's disease

Insufficient apoptosis:

Cancer

Autoimmune diseases



Breast cancer cell

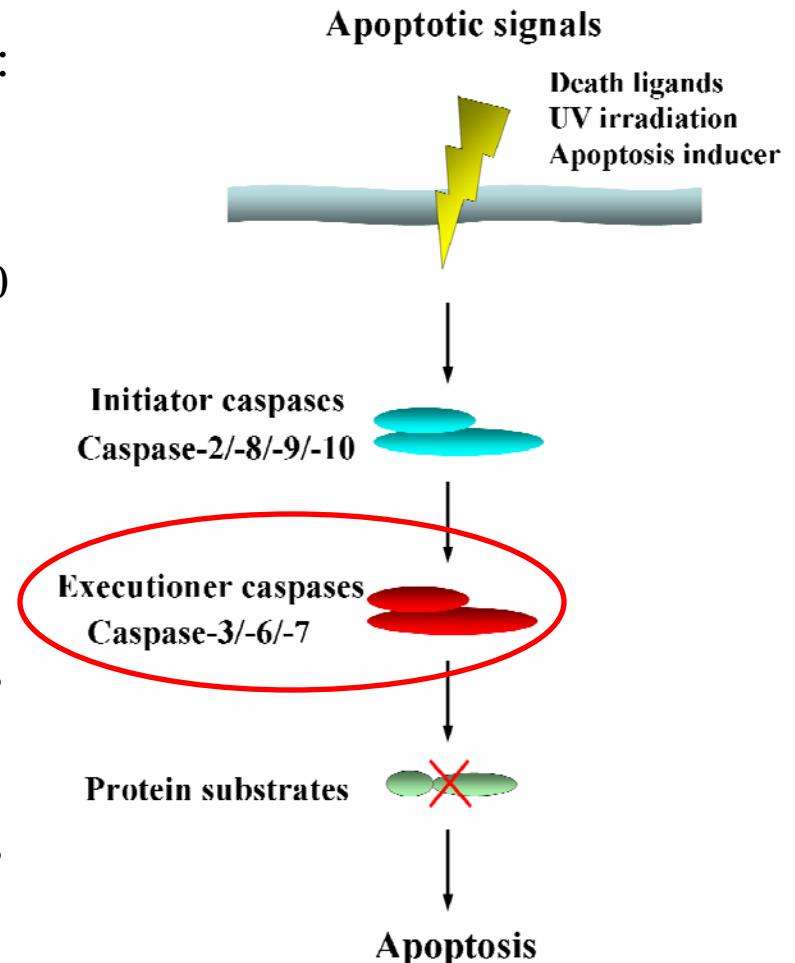
Caspases: Major Players in Apoptosis

Caspases are **cysteine proteases** involved in:

- Inflammatory responses — Caspase 1, 4, 5
- Apoptosis
 - Initiators — Caspase 2, 8, 9, 10
 - Executioners — **Caspase 3, 6, 7**

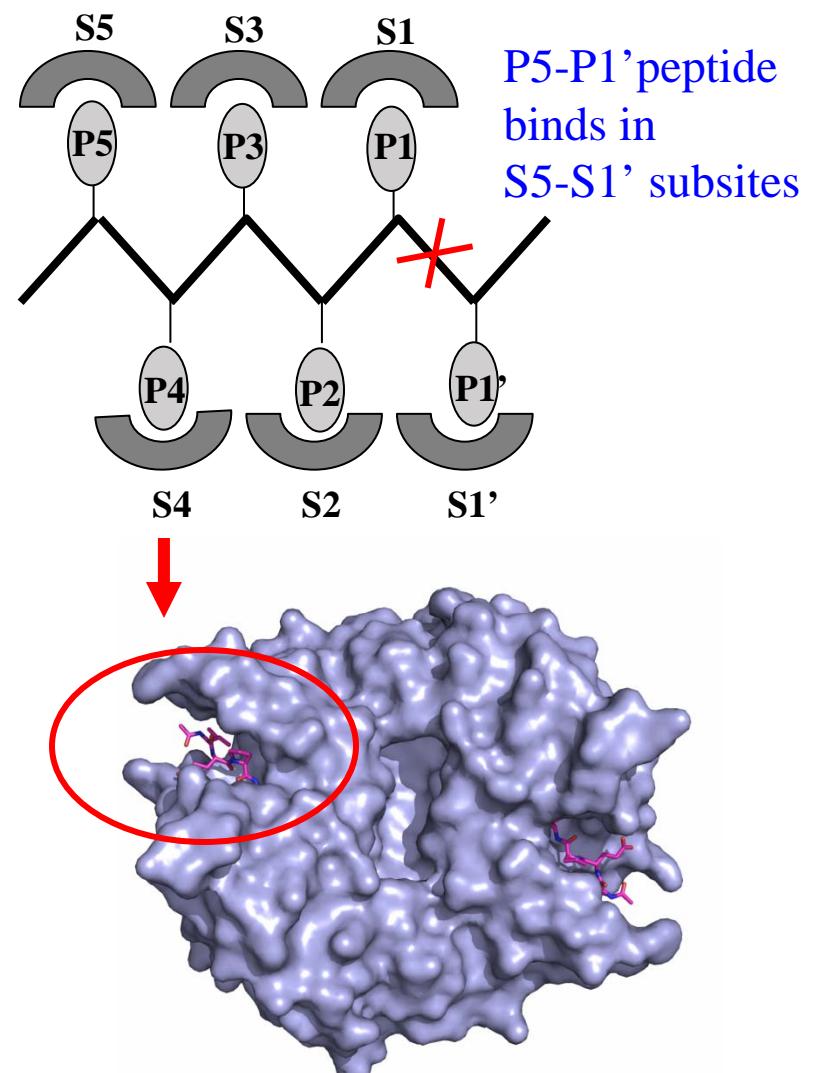
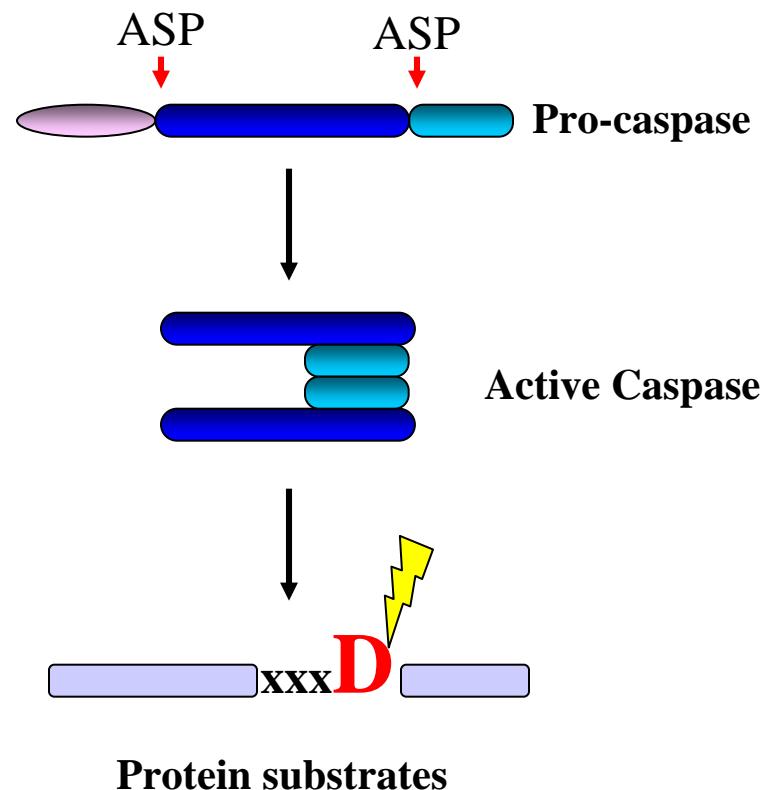
Goal of Research:

- To help identify caspase-mediated pathways leading to cell death
- To assist in structure-guided design of drugs



Caspases Recognition of Substrates

Caspases recognize **tetrapeptide sequences** and hydrolyze the peptide bond after **Asp (D)** in protein substrates

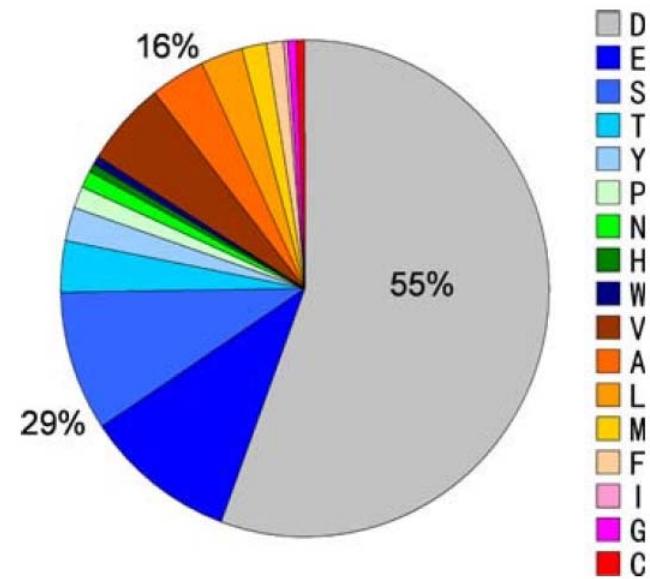


Known Substrate Preferences of Caspases

Preferred P4-P1		
Inflammatory caspases	caspases 1,4,5	WEHD
Initiators of apoptosis	caspase 2 caspases 8,9,10	DEHD LEXD
Executioners of apoptosis	caspase 6 caspase 3,7	VEHD DEVD ★

(Thornberry et al., 1997)

Caspase-3 binds diverse **P4 residues** in substrates



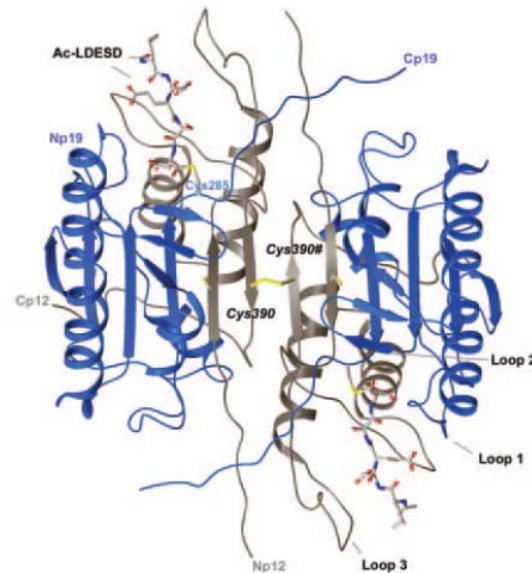
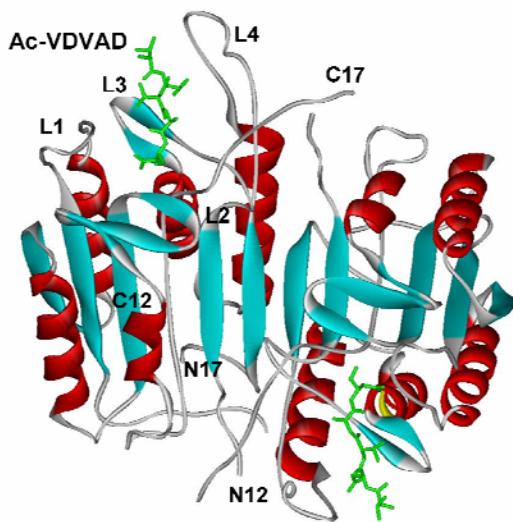
Occurrence of amino acids at P4 in 183 protein substrates of caspase-3 (*data from Fischer et al, Cell Death Differ, 2003*)

Question: Do “executioner” Caspase-3 and -7 differ in their substrate specificity beyond the “canonical” DEVD sequence?

1. Johnson Agnuswamy et al. (2007) *The FEBS Journal*.
2. Bin Fang et al. (2009) *Apoptosis*.

Previous Studies on Caspase Recognition of P5

- Schweizer et al. reported a P5 binding pocket in the crystal structure of Caspase-2 with Ac-LDESD-CHO¹.



- Fang et al. found the P5 binding site in crystal structure of Caspase-3 with Ac-VDVAD-CHO².

1. Andreas Schweizer et al. (2003) *J. Biol. Chem.*

2. Bin Fang et al. (2006) *J. Mol. Biol.*

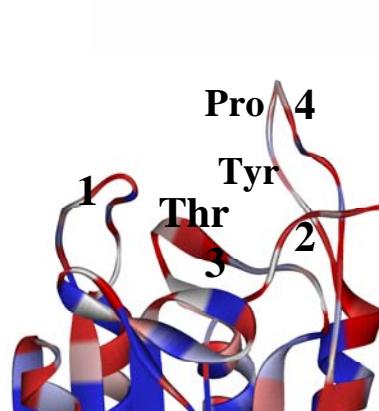
What is the P5 preference of
executioner caspases?

Caspases Differ in S5 Subsite

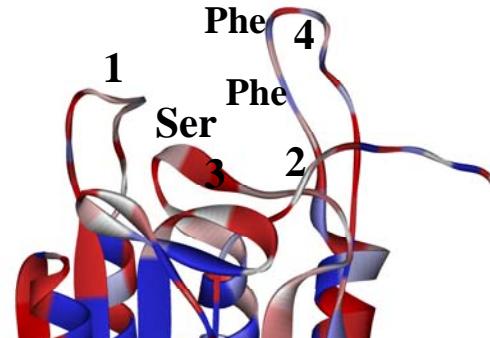
Loop-3

*	20	*	40	*	60
Caspase-2 : GTAAMRN T KRG S WY I E A LAQVF S ERA-CDMHVADMLVKVNALIKDR-EG Y AP G TEF H R C E					
Caspase-3 : GYYSWRN S KDG S WFI Q SL C AM I K Q YA-DK L EFM H IL T RVNRKVATE F E S F S E DATFHAK K Q					
Caspase-6 : GYYSHRE T VNG S WY I QDL C EM I G K YG-SS L EFT E LL T LVNRKV S QRR V D F C K DPSAIGKK Q					
Caspase-7 : GYYSWRS P GRG S WFV Q AL C SI E EH G -KD L E I M Q IL T RVND R VARH F E S Q S D DPH F HEKK Q					
Caspase-8 : NCVSYRN P AEG T WY I Q S LC Q SI R RCPRGDD I L T IL E VNYEV S NK-----DDKKNM G Q					

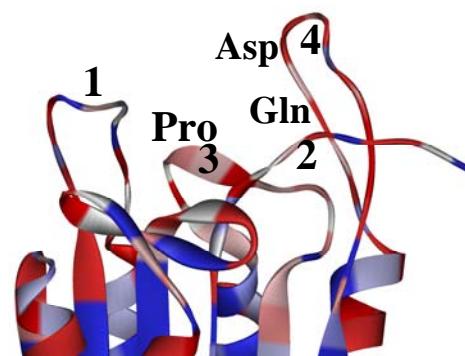
Loop-4



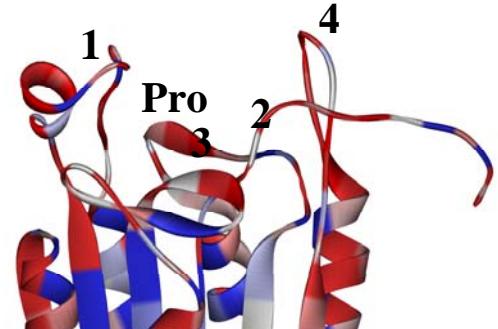
Caspase-2



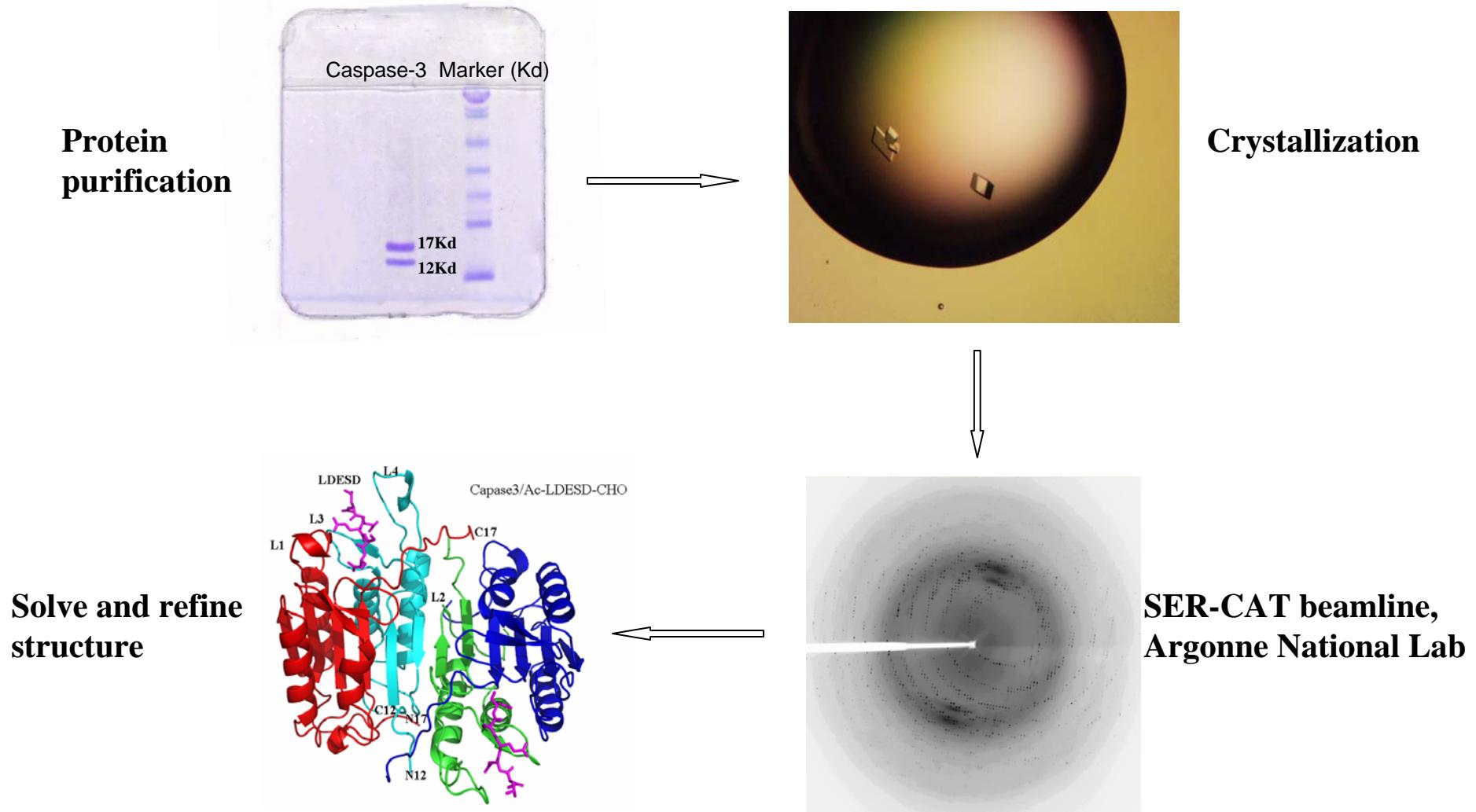
Caspase-3



Caspase-8



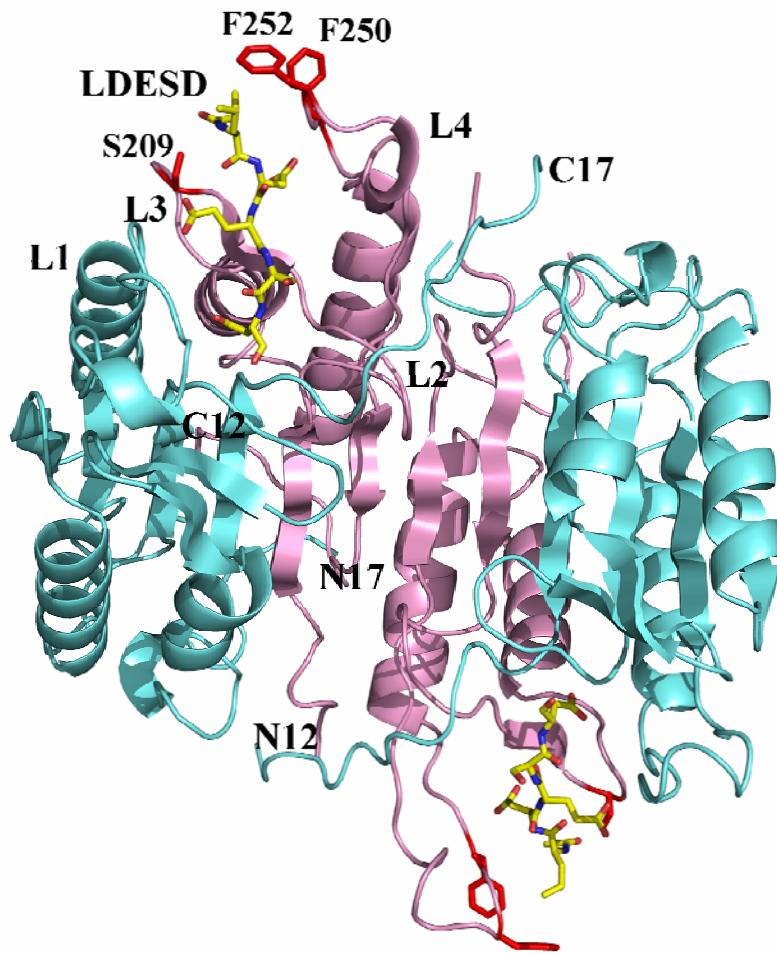
Crystallization of Caspase-3/-7 with Ac-LDESD-CHO



Structure of Caspase-6/Ac-LDESD-CHO was constructed with molecular modeling program AMMP¹.

1.Harrison RW . (1993) J. Comp. Chem.

Crystallographic Data for Caspase-3/-7 with Ac-LDESD-CHO

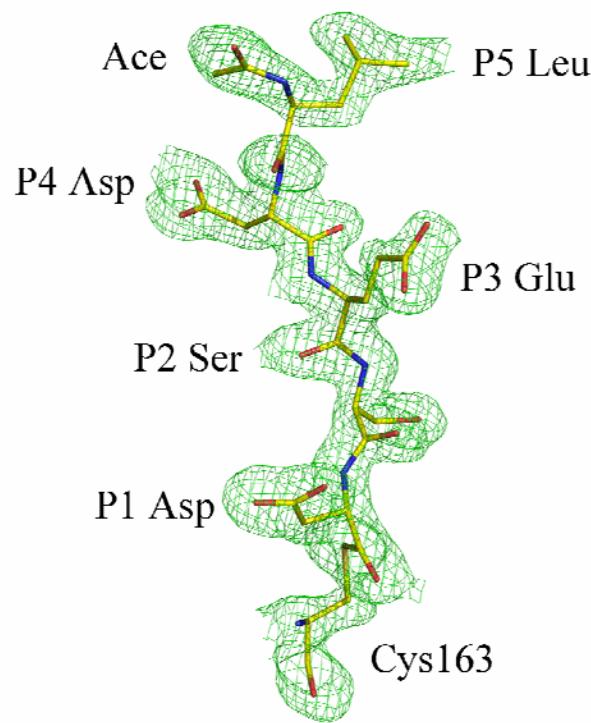


	Caspase3/LDESD	Caspase7/LDESD
Space group	P 2₁2₁2₁	P3₂1
a (Å)	67.37	88.64
b (Å)	93.56	88.64
c (Å)	97.62	187.71
Resolution (Å)	1.61	2.45
Total observation	358,844	175,449
Unique reflections	72,895	28,712
Completeness (%)	90.1 (63.9)*	90.3(57.3)
<I/σ(I)>	15.3(2.4)	18.5(2.7)
R-sym(%)	7.5(41.6)	9.5(38.9)
R-work (%)	17.4	20.1
R-free (%)	22.3	24.8
r.m.s. deviations		
Bond length (Å)	0.009	0.006
Angles	0.029 (Å)	1.35 (°)

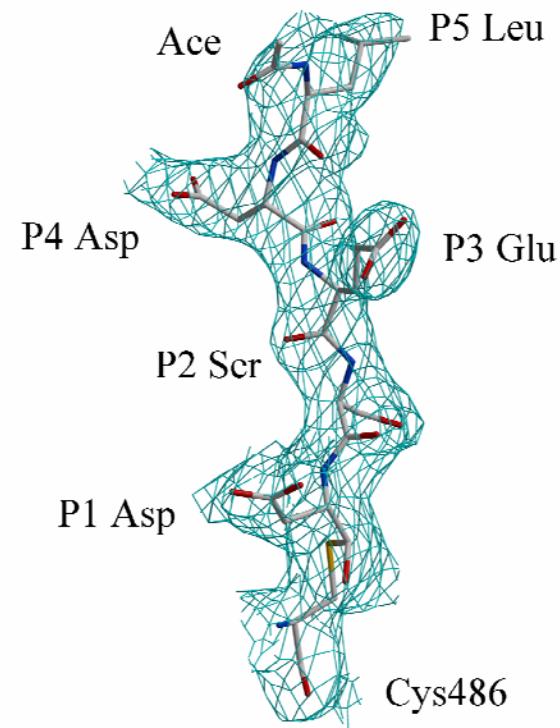
* Values in parentheses are given for the highest resolution shell

Fu et al. (2008) Apoptosis.

2Fo-Fc Electron Density for Ac-LDESD-CHO

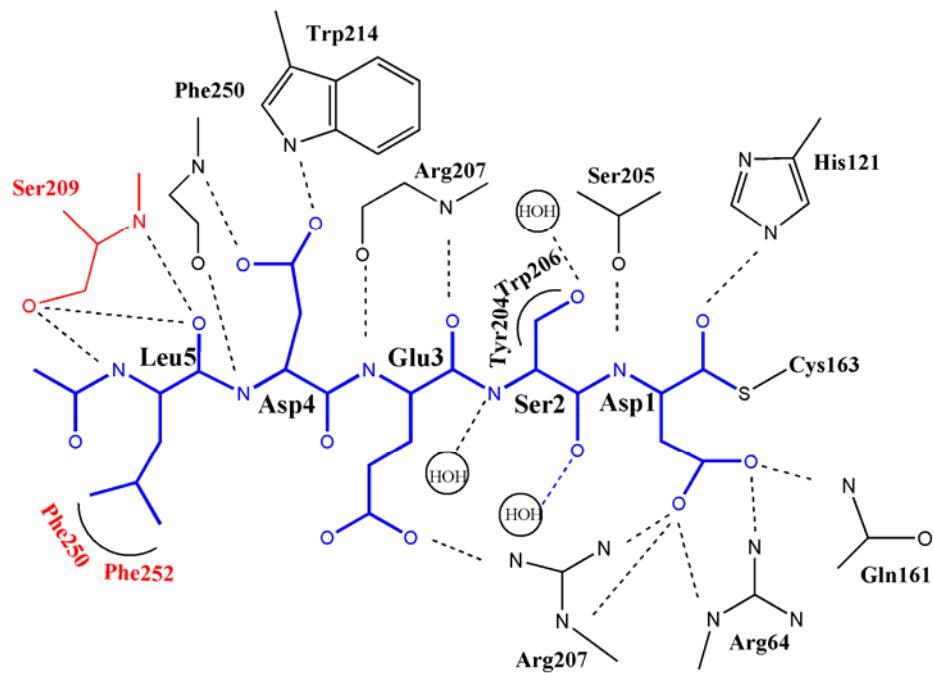


Caspase-3/LDESD
(crystal structure 1.6 Å)

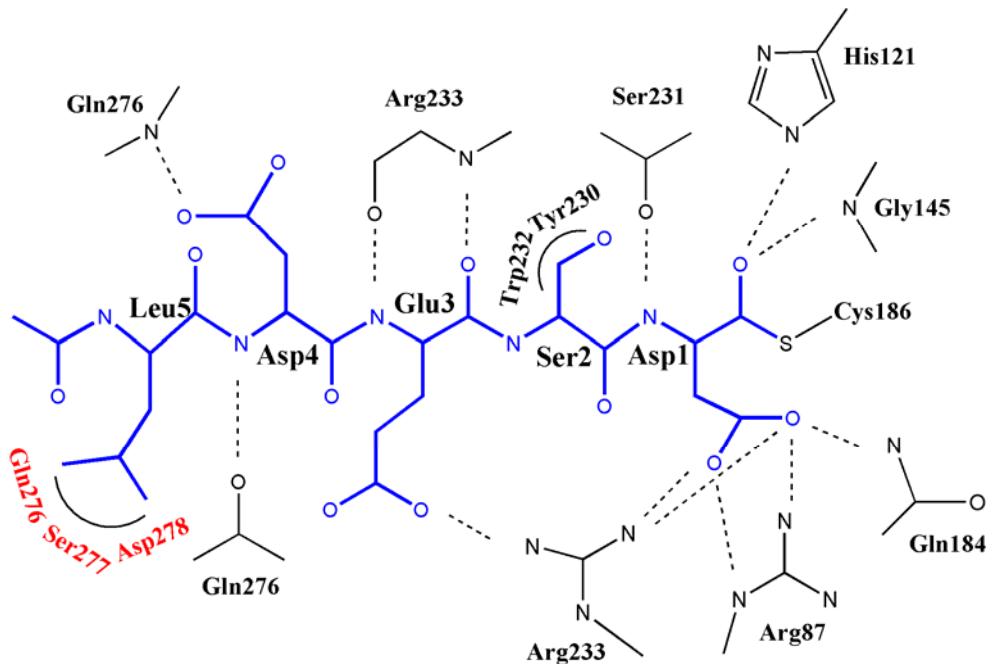


Caspase-7/LDESD
(crystal structure 2.4 Å)

Caspase-3 and -7 Recognition of Pentapeptides

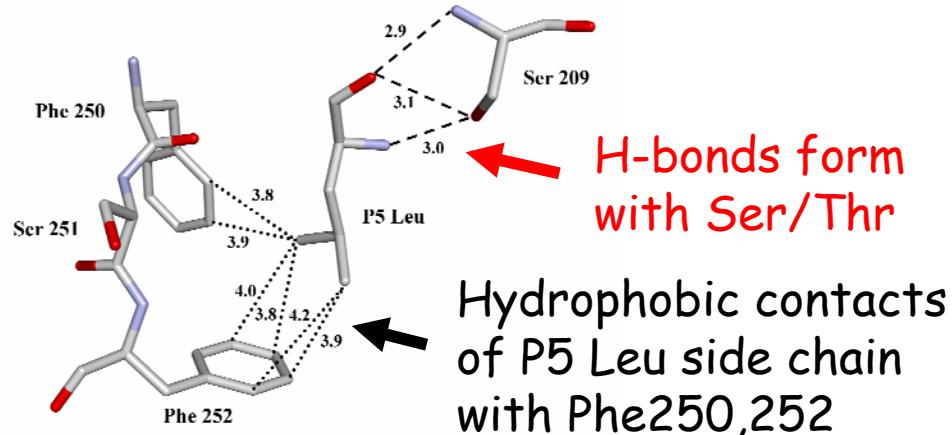


Caspase-3/LDESD
(crystal structure 1.6 Å)

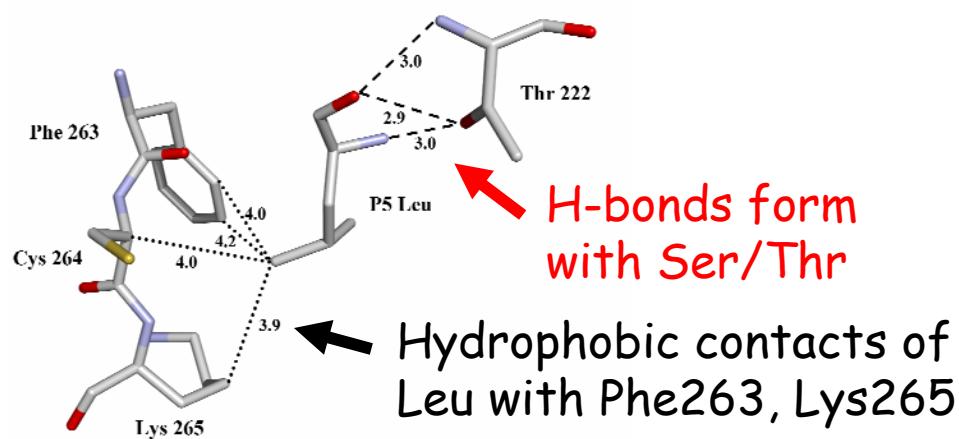


Caspase-7/LDESD
(crystal structure 2.4 Å)

Interactions of P5 Leu in S5 subsite of caspases

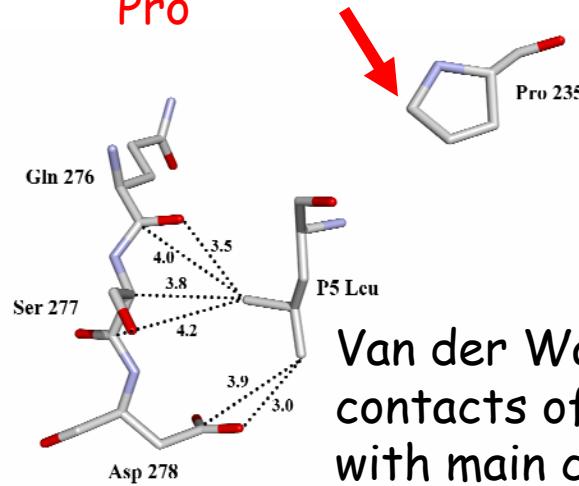


Caspase-3/LDESD
(crystal structure 1.6 Å)



Caspase-6/LDESD (model)

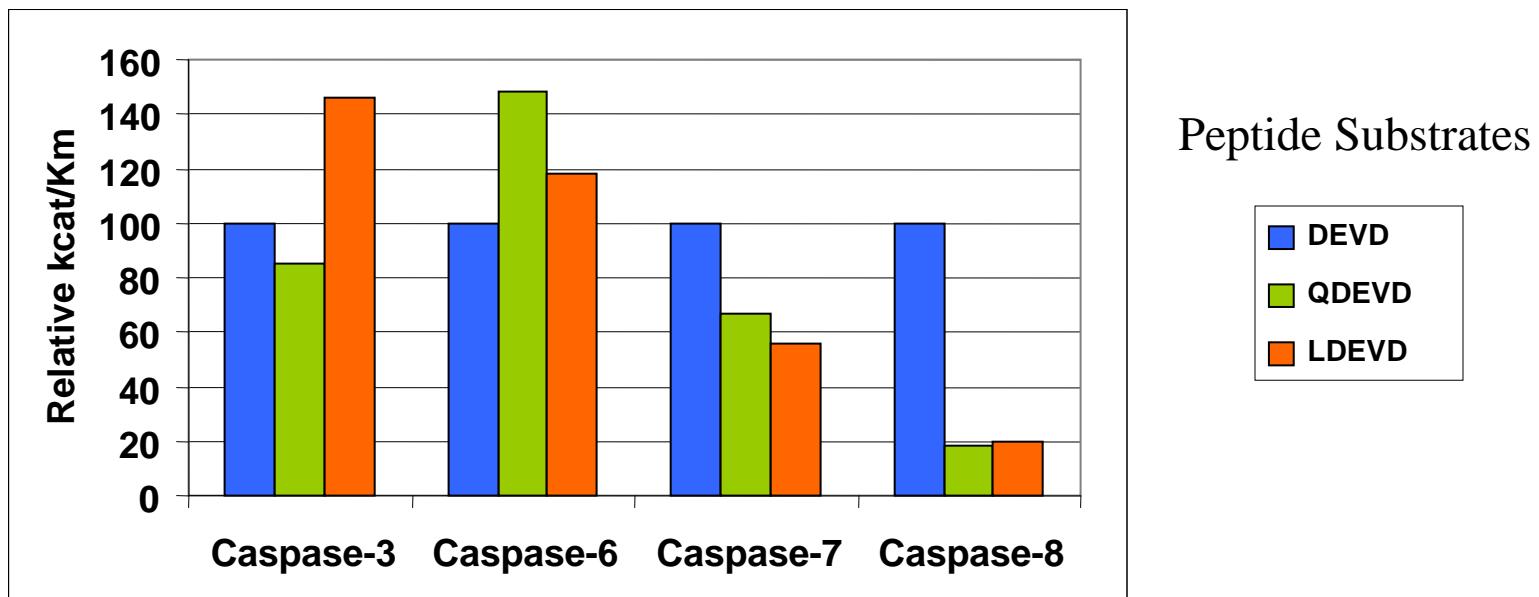
No H-bonds can form with Pro



Van der Waals contacts of Leu with main chain and polar Ser277, Asp278

Caspase-7/LDESD
(crystal structure 2.45 Å)

Caspases Differ in P5 Specificity



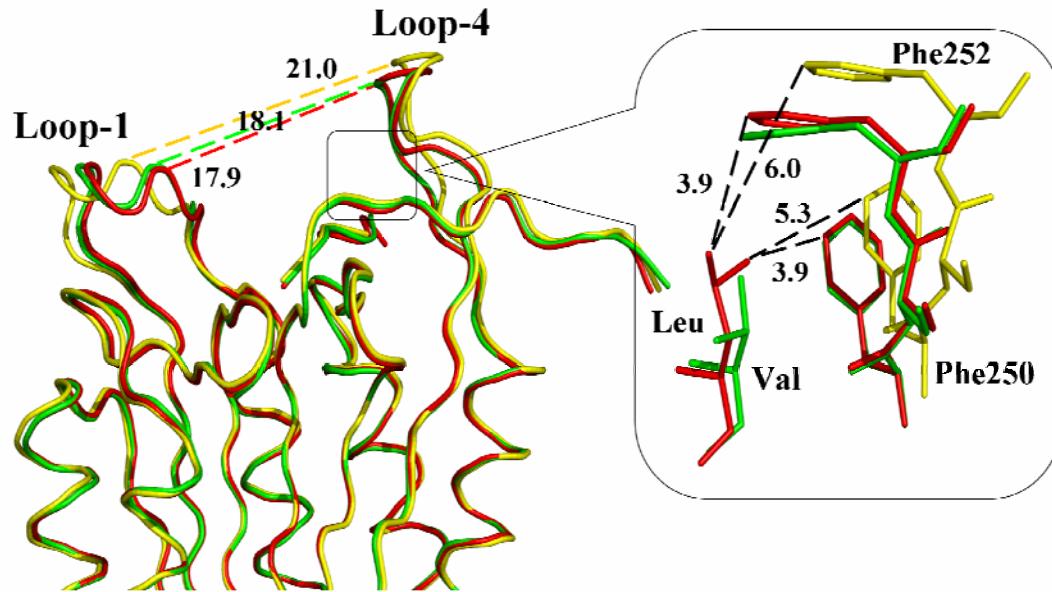
Caspase-3 prefers hydrophobic P5

Caspase-6 prefers polar over hydrophobic P5

Caspase-7 prefers tetrapeptide DEVD over pentapeptides

Caspase-8 strongly prefers tetrapeptide DEVD over pentapeptides

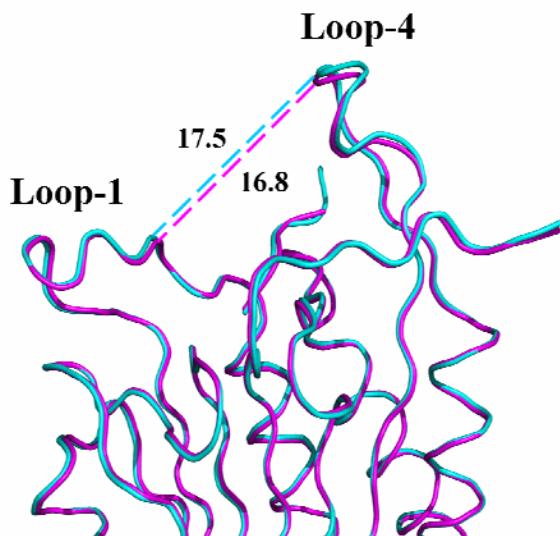
Conformational Change in Caspase-3/-7 with Pentapeptide



Induced-fit mechanism

Caspase-3 in complex with
DEVD, **VDVAD** and **LDESD**.

Caspase-7 shows no
conformational change
with **DEVD** and **LDESD**.



Conclusions

- Executioner caspases differ in their recognition of P5 residue in substrates.
 - Caspase-3 prefers hydrophobic P5 residues.
 - Caspase-6 prefers polar P5 residues.
 - Caspase-7 prefers tetrapeptide over pentapeptide.

- Caspase-3 recognizes pentapeptides by an induced-fit mechanism.

- Caspases with Ser/Thr in their loop-3 of S5 subsite recognize P5 residue in substrates, like caspase-2 , -3 and -6 (possibly 4 and 5).
- Caspases with Pro in their loop-3 of S5 subsite do not recognize P5 residue in substrates, like caspase-7 and -8 (possibly 1 and 9).



Acknowledgements



Dr. Irene Weber
Dr. Robert Harrison

Dr. Johnson Agnieszka
Dr. Alexander Chumanovich
Yuan-Fang Wang
Ting-Yi Chiu
Bin Fang
Chen-Hsiang Shen
Ying Zhang
Xiaxia Yu



Georgia Cancer Coalition
National Institutes of Health
Molecular Basis of Disease Program, GSU