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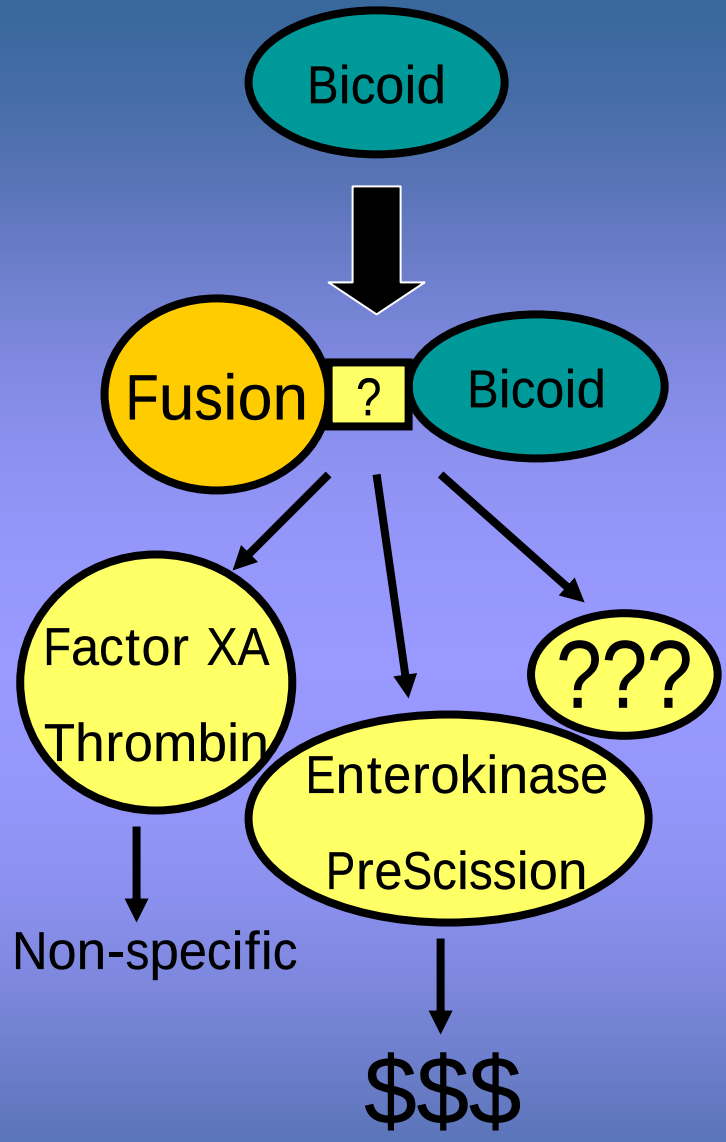
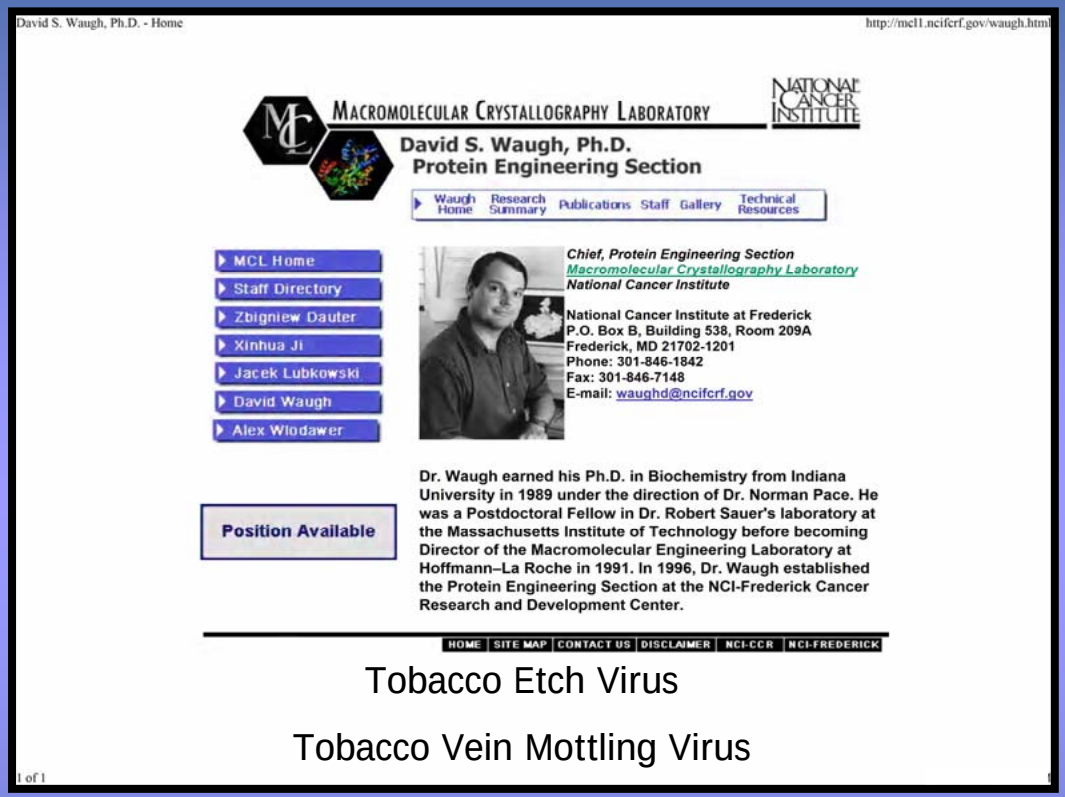




Tobacco Etch Virus

Tobacco Vein Mottling Virus

http://mcl1.ncifrcrf.gov/waugh.html

(1) Why TEV/TVMV?

(2) Obtaining the TEV or TVMV Proteases

(3) Production

(4) Cleavage of your fusion protein

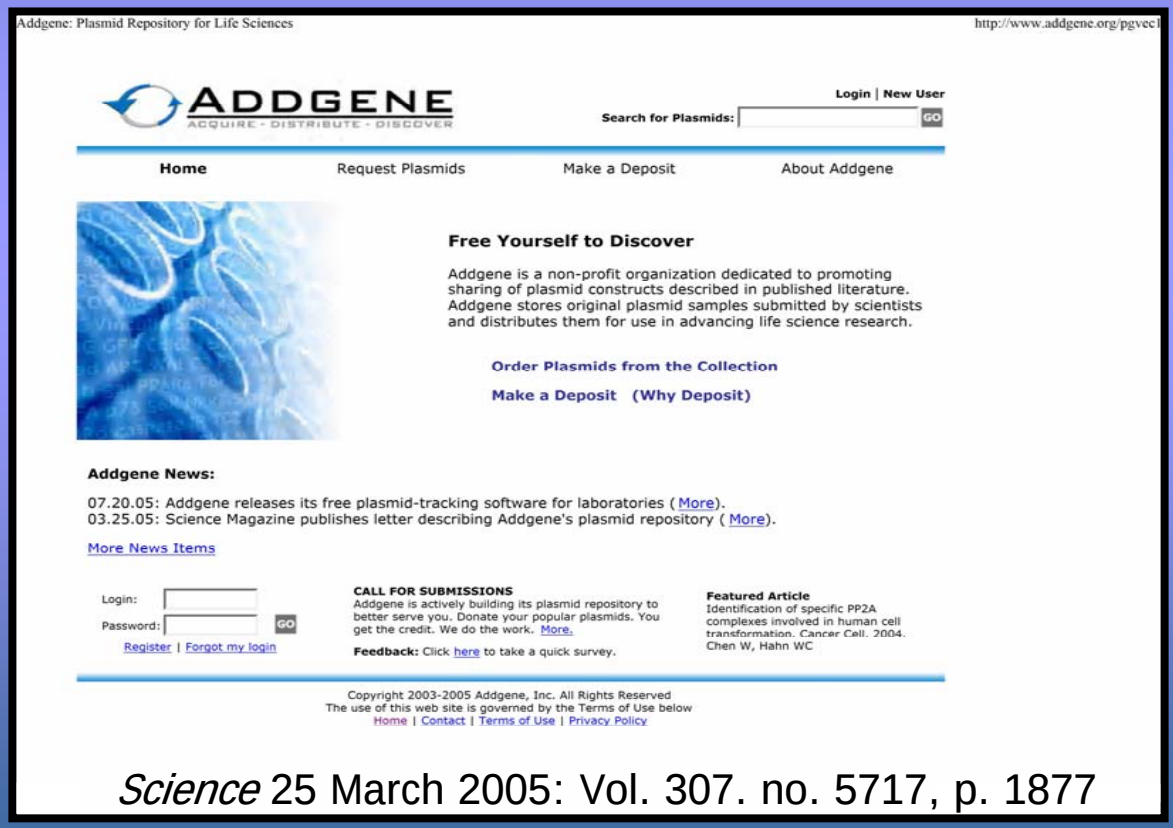
(5) Novel Applications/ Just For Fun

- Easy to Purify in Soluble form in Large Quantities
- Highly Specific Cleavage Sites (7aa)
- Inexpensive to Produce
- Leaves only one non-native N-terminal glycine on your protein of interest
- Easy to remove from your protein after cleavage is complete

How to Obtain the TEV and/or TVMV Proteases

Obtaining the TEV/TVMV Proteases for your Lab

http://addgene.org



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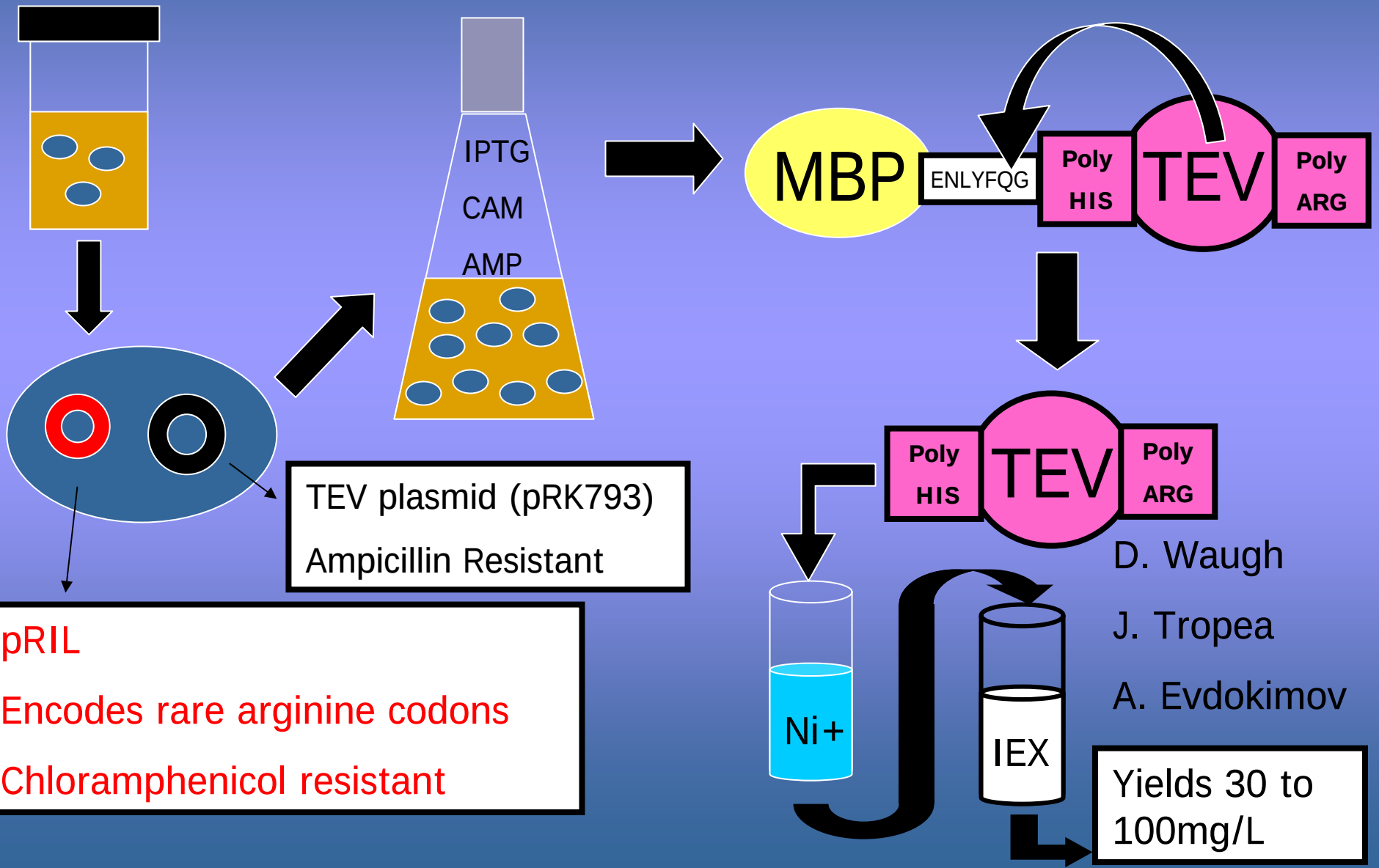
Production of the Protease in your Lab

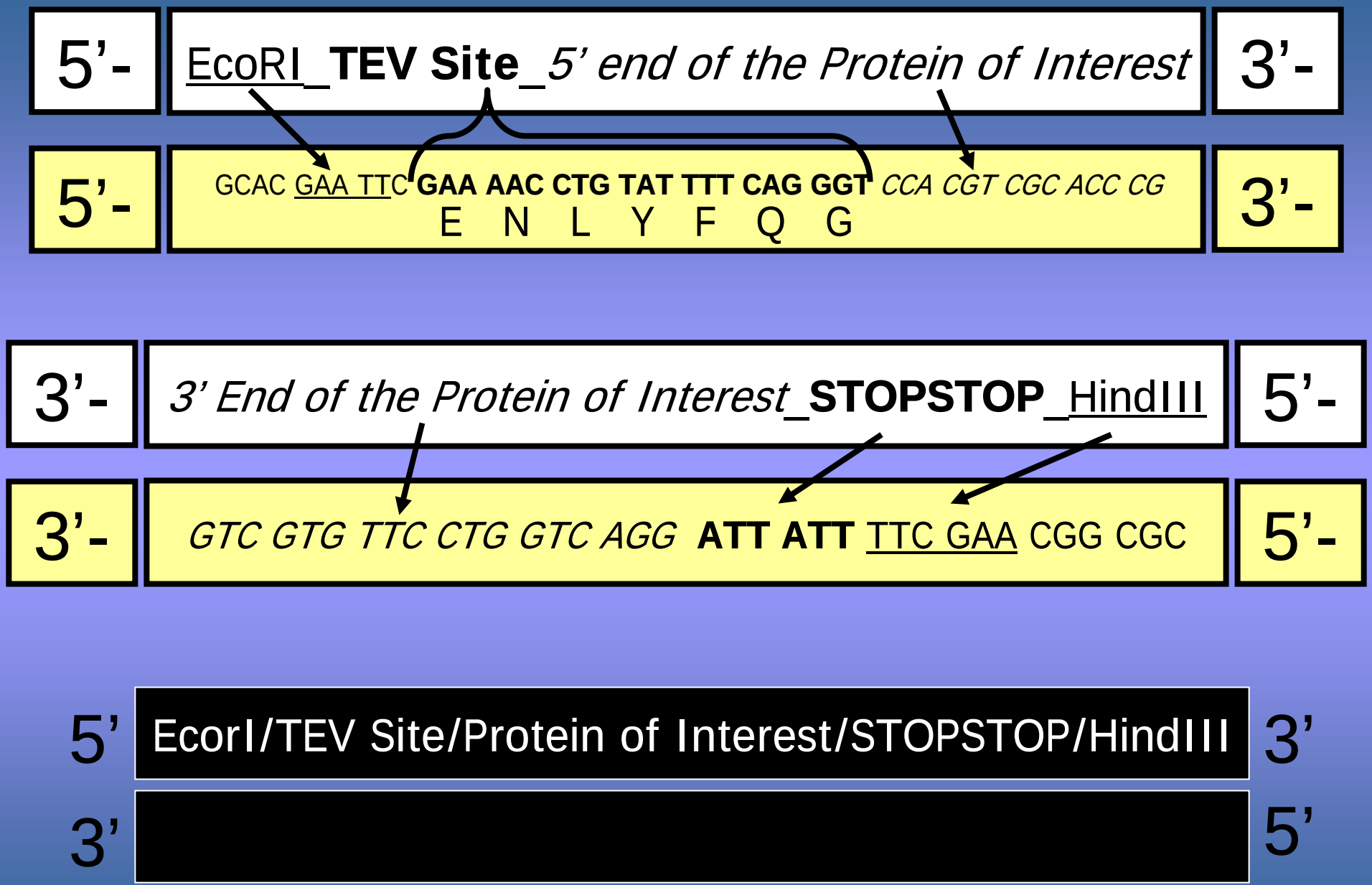
Production of the TEV/TVMV Protease



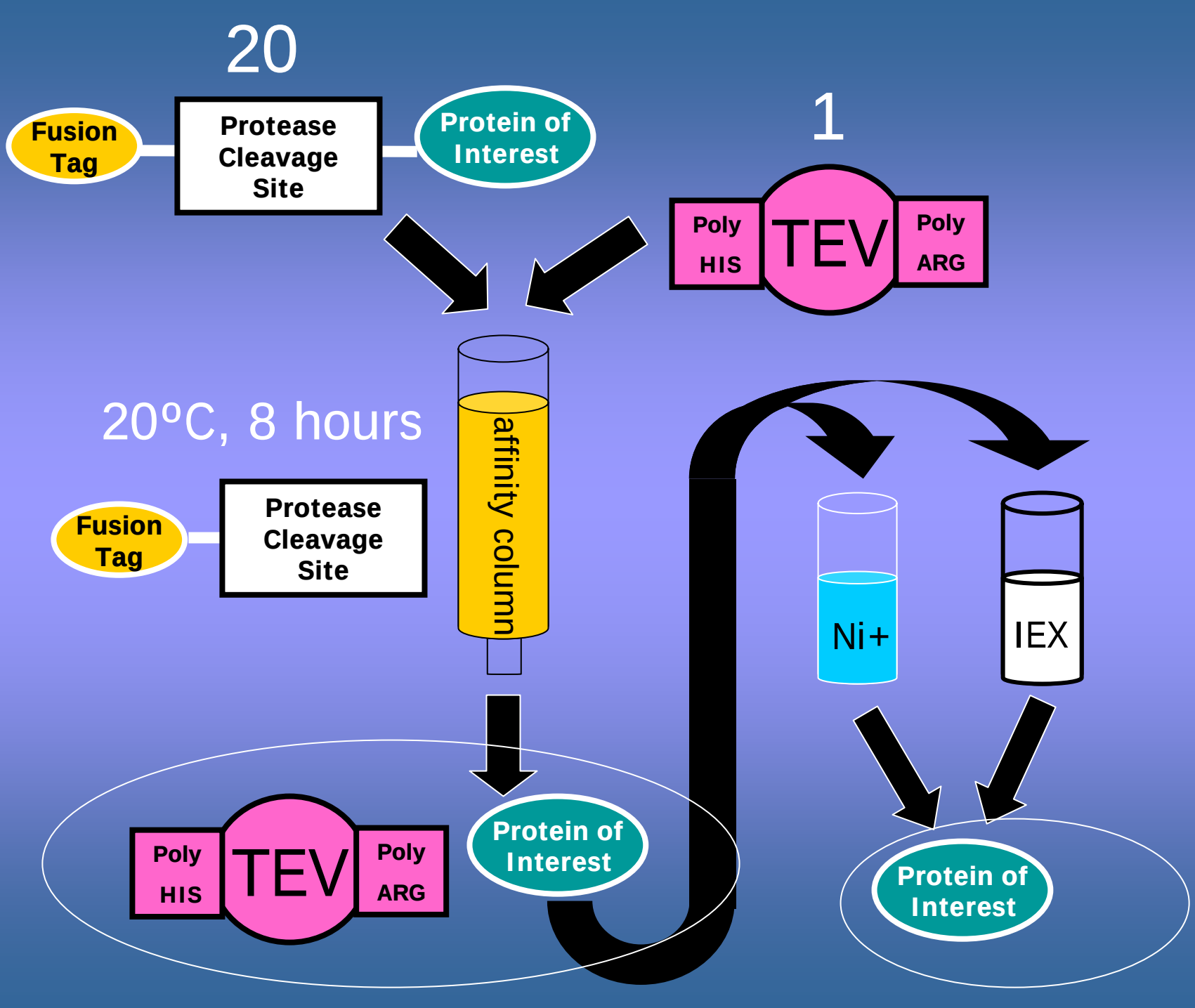
Special Notes on TEV Production

- Sonication of the cell pellets containing the TEV protease may result in inactivation
- Recommended reagents, buffers, and more detailed protocols can be found on the website of Dr. Waugh
- Most buffers contain minimum 5% glycerol; TEV is stored in buffer containing 40% glycerol
- TEV is stable at -80ºC for 1 year

Incorporation of the TEV cleavage site into your system


5'-
EcoRI_TEV Site_5' end of the Protein of Interest
3'-
5'-
GCAC GAA TTC GAA AAC CTG TAT TTT CAG GGT CCA CGT CGC ACC CG
E N L Y F Q G
3'-
3'-
3' End of the Protein of Interest_STOPSTOP_HindIII
5'-
3'-
GTC GTG TTC CTG GTC AGG ATT ATT TTC GAA CGG CGC
5'-
5'
EcorI/TEV Site/Protein of Interest/STOPSTOP/HindIII
3'
3'
5'

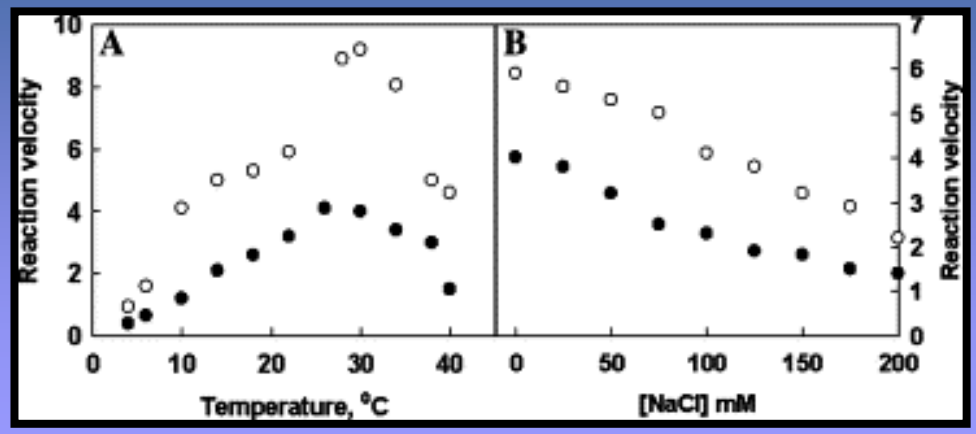
Using the TEV or TVMV Protease in your System


20
Fusion Tag
Protease Cleavage Site
Protein of Interest
1
Poly HIS
TEV
Poly ARG
20°C, 8 hours
affinity column
Fusion Tag
Protease Cleavage Site
Ni+
IEX
Poly HIS
TEV
Poly ARG
Protein of Interest
Protein of Interest

Reaction Optimization

Optimum T = 30ºC

Optimum [salt] = 0mM



TEV/TVMV Cross-reactivity

And Kinetic Parameters

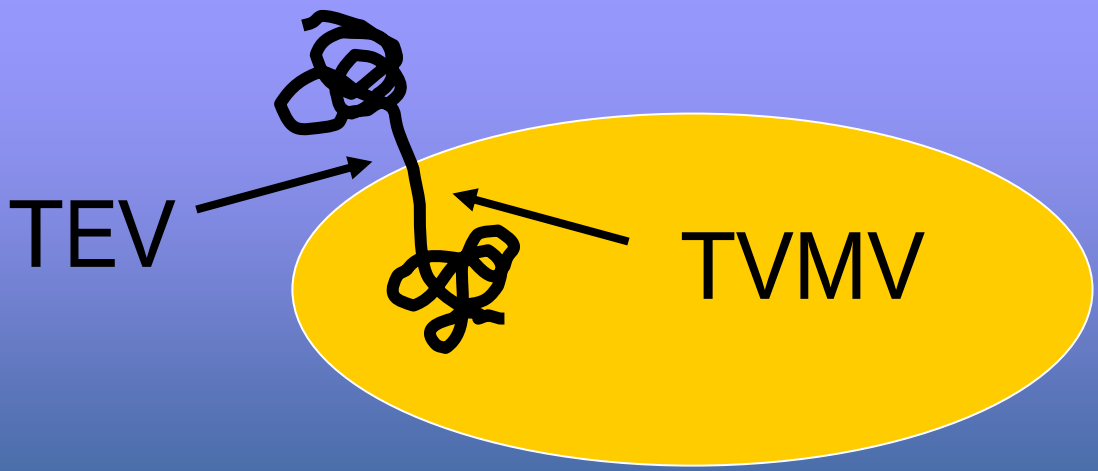
Kinetic parameters for TVMV and TEV proteases

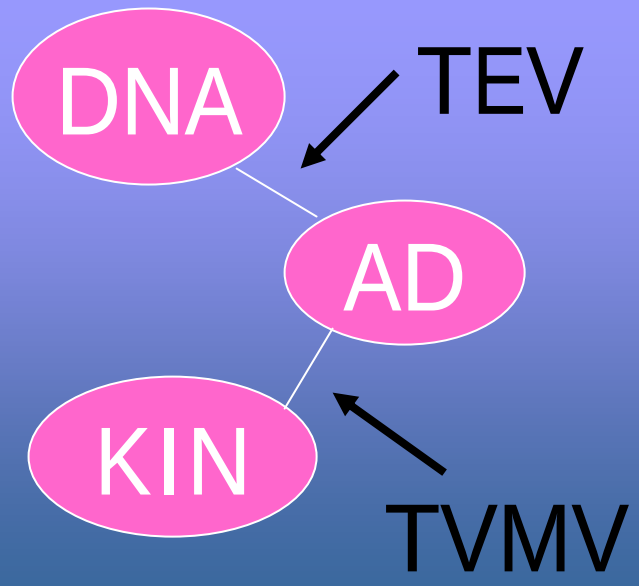
Substrate	Enzyme	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($mM^{-1}s^{-1}$)
TENLYFQ/SGTRR	TEV	0.061 ± 0.010	0.16 ± 0.01	2.62 ± 0.46
TETVRFQ/SGTRR	TVMV	0.065 ± 0.009	0.07 ± 0.01	1.08 ± 0.17
TENLYFQ/SGTRR	TVMV	Not cleaved		
TETVRFQ/SGTRR	TEV	Not cleaved		

Nallamsetty, et. al, Prot. Exp. Purif., 2004

Just For Fun


Fusion Tag 1
TEV
Fusion Tag 2
TVMV
Your Protein


TEV
TVMV


DNA
TEV
AD
KIN
TVMV