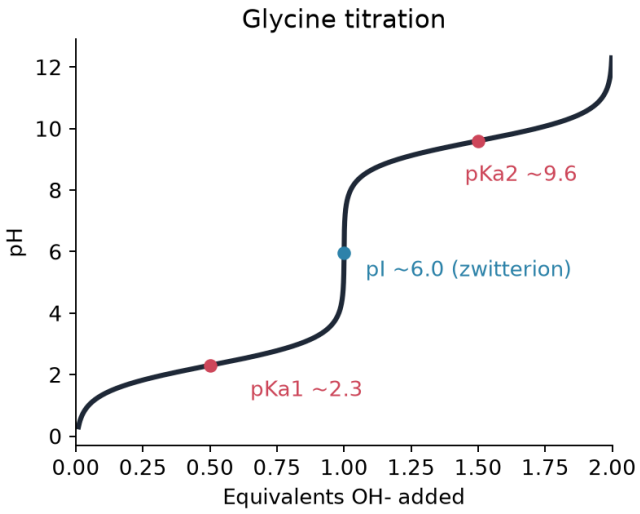
Amino Acids, Proteins and Enzymes: Cheat Sheet

The 20 amino acids by side chain

3-letter	1-letter	Side chain notes	Charge at pH 7.4
NONPOLAR ALIPHATIC			
Gly	G	achiral, smallest	0
Ala	A	methyl	0
Val	V	branched	0
Leu	L	branched	0
Ile	I	branched, 2 chiral C	0
Met	M	thioether, start	0
Pro	P	ring on N, kinks chain	0
AROMATIC			
Phe	F	hydrophobic ring	0
Trp	W	largest, UV 280 nm	0
Tyr	Y	phenol, pKa 10.1, P-site	0 (polar)
POLAR UNCHARGED			
Ser	S	OH, P-site, O-glyc	0
Thr	T	OH, P-site, O-glyc	0
Cys	C	SH, pKa 8.3, S-S	0
Asn	N	amide, N-glyc	0
Gln	Q	amide	0
ACIDIC (-)			
Asp	D	pKa 3.9	-1
Glu	E	pKa 4.3	-1
BASIC (+)			
Lys	K	pKa 10.5	+1
Arg	R	pKa 12.5, guanidinium	+1
His	H	pKa 6.0, imidazole	~0 (few % +)

Titration and pI



- Neutral aa: pI = (pKa1 + pKa2)/2, about 6
- Acidic aa: average the two acidic pKas
- Basic aa: average the two basic pKas
- pH < pI: net +; pH > pI: net -

Protein structure

Level	What / forces
1°	Sequence; peptide bonds (planar, trans, partial double bond)
2°	Alpha helix (i to i+4, 3.6/turn), beta sheet; backbone H bonds. Pro breaks, Gly flexible
3°	Overall fold: hydrophobic effect, H bonds, salt bridges, van der Waals, disulfides
4°	Multiple subunits (Hb alpha2beta2)

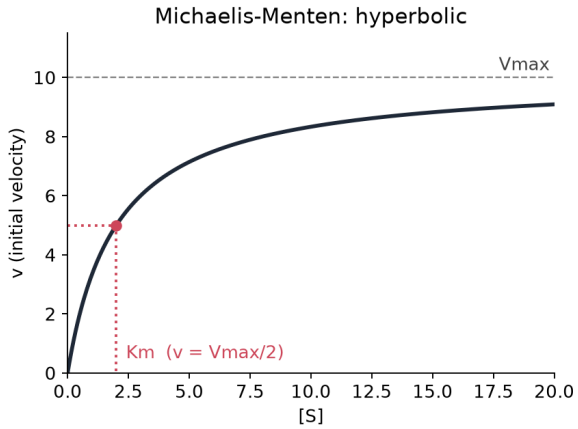
Denaturation

- Heat, extreme pH, urea, guanidinium: break noncovalent bonds
- SDS: unfolds, coats with negative charge (SDS-PAGE by mass)
- BME / DTT: reduce disulfides
- 1° structure survives

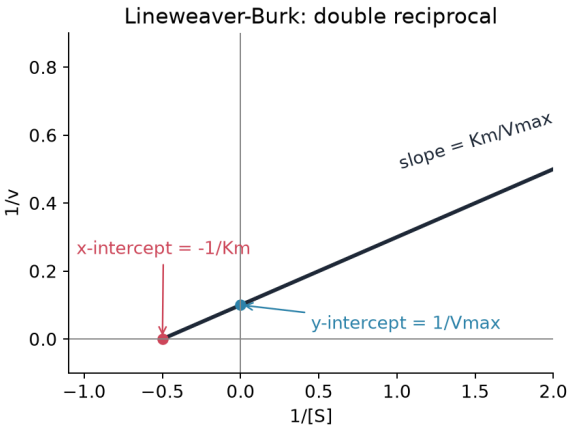
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Enzyme kinetics

Michaelis-Menten



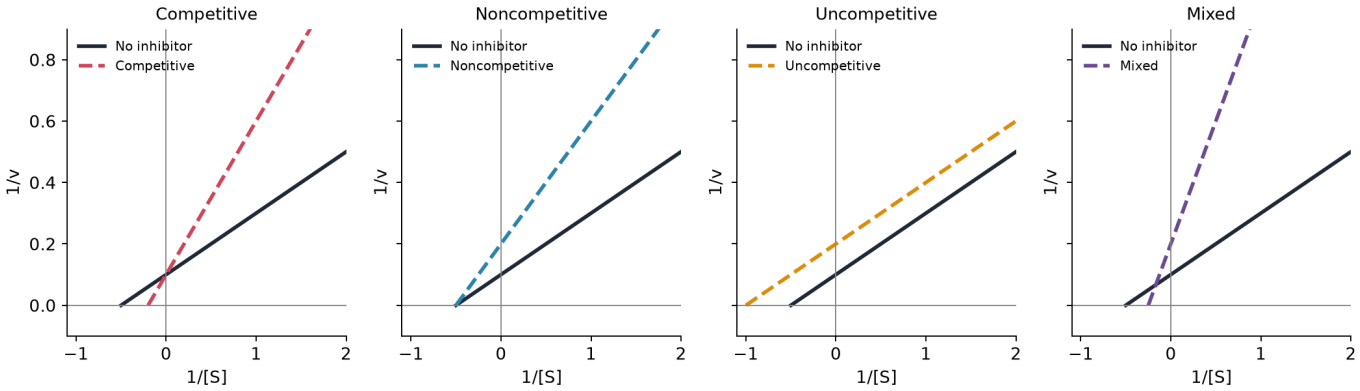
Lineweaver-Burk



v = Vmax[S]/(Km + [S]) Vmax = kcat[E]total Km = [S] at Vmax/2 (low Km, high affinity) efficiency = kcat/Km [S] = 3Km gives 75% Vmax, 9Km gives 90%.

Inhibition summary

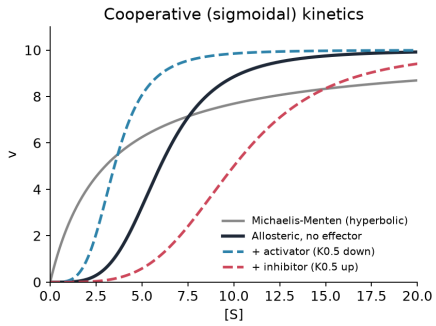
Type	Binds	Km	Vmax	Lineweaver-Burk	Overcome by [S]?
Competitive	E (active site)	up	same	cross at y axis	Yes
Noncompetitive	E and ES equally	same	down	cross at x axis	No
Uncompetitive	ES only	down	down	parallel lines	No
Mixed	E and ES unequally	up or down	down	cross off axes	Partly
Irreversible	Covalent	same	down	like noncompetitive	No



Enzyme classes

Class	Does
Oxidoreductase	redox
Transferase	moves group (kinase)
Hydrolase	cleaves with water
Lyase	cleaves/forms, no water or redox
Isomerase	rearranges one molecule
Ligase	joins, uses ATP
Translocase (EC 7)	moves ions/molecules across membranes

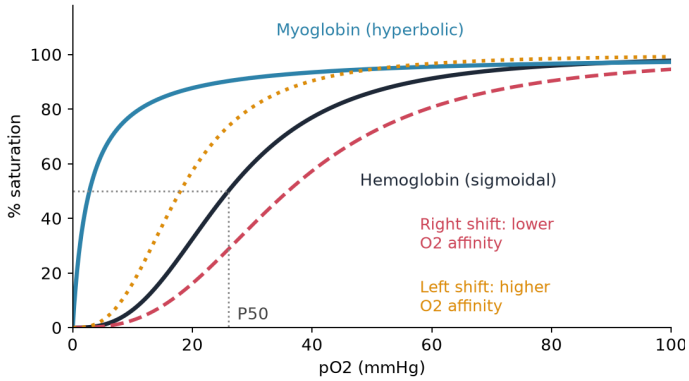
Allostery and regulation



- Hill n > 1: positive cooperativity (Hb about 2.8, Mb 1)
- Activator shifts left, inhibitor right (K0.5)
- Zymogens, phosphorylation, feedback inhibition

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Hemoglobin, motors, fibrous proteins and methods



	Myoglobin	Hemoglobin
Chains	1	4 (alpha2 beta2)
Curve	hyperbolic	sigmoidal
P50	about 2.8 mmHg	about 26 mmHg
Hill n	1	about 2.8

- **Right shift (unload):** H+, CO2, heat, 2,3-BPG
- **Left shift:** lower H+, CO2, temperature, or BPG; HbF; CO
- O2 binds heme Fe2+ only

Fibrous, motor and binding proteins

Protein	Key facts
Collagen	triple helix, Gly-X-Y, Hyp needs vitamin C
Keratin	alpha helix, disulfides
Elastin	cross-linked random coil, stretches
Myosin	walks on actin, ATP
Kinesin	microtubule plus end (to axon tip)
Dynein	microtubule minus end
Antibody	2 heavy + 2 light, Fab binds, Fc effector

Cofactors

Vitamin	Coenzyme
Niacin	NAD+
Riboflavin	FAD
Thiamine	TPP
Pantothenate	CoA
Pyridoxine (B6)	PLP

Protein isolation and analysis

Method	Separates by	Notes
SDS-PAGE	mass	SDS denatures and gives uniform negative charge; small proteins run farthest; BME breaks disulfides
Native PAGE	charge, size, shape	keeps the fold
Isoelectric focusing	pI	protein stops where pH = pI
Size exclusion	size	large proteins elute first
Ion exchange	net charge	elute with salt or pH change
Affinity	specific binding	immobilized ligand
Western / ELISA	antibody detection	specific protein or antigen

Purification: specific activity = units/mg. Fold purification = final specific activity / starting specific activity. Yield = final total units / starting total units. A280 reads Trp and Tyr. Bradford (Coomassie dye) and BCA (Cu reduction) are colorimetric assays.

Henderson-Hasselbalch: pH = pKa + log([A-]/[HA]). pH below pKa: protonated. pI: neutral aa = average of 2 backbone pKas; acidic = average of 2 lowest; basic = average of 2 highest. Km at v = Vmax/2; K0.5 for allosteric enzymes.

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