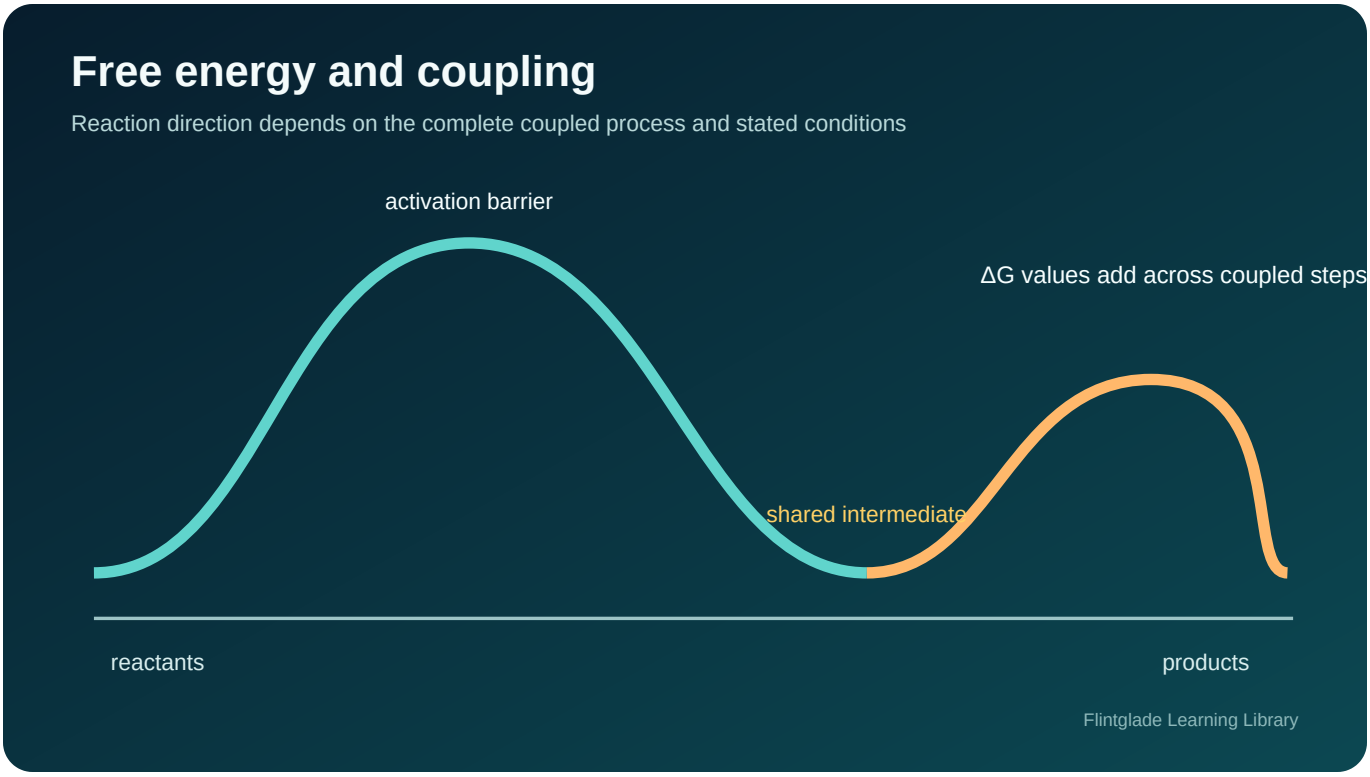
Thermodynamic Direction and Reaction Coupling

Flintglade Learning Library · General Biochemistry

Audience: Readers learning to reason about free energy, equilibrium, concentration effects, and coupled reactions.

MCAT connections: This guide can assist study of energetics and metabolism while keeping general biochemistry as its primary frame.



About this edition: This standalone edition organizes the material for independent reading with clear navigation, reusable reasoning frameworks, and print-ready presentation.

Learning goals

- Separate standard-state values from cellular free-energy changes
- Use reaction quotients to reason about direction
- Explain how coupling and product removal reshape pathway behavior

Navigate this guide

The One Rule That Governs Everything

Every spontaneity analysis reduces to one thing: **ΔG**. In many qualitative examples, no calculation is needed. Instead, you need trained intuition. This guide gives you that intuition by teaching you to *see* what's happening thermodynamically just by looking at a reaction.

$$\Delta G = \Delta H - T\Delta S$$

In biochemistry, we care about **ΔG°'** (standard biochemical conditions, pH 7, 25°C, 1 M concentrations) unless told otherwise. Under **cellular conditions**, actual concentrations shift ΔG — sometimes flipping the sign entirely.

The Three Questions (Ask These Every Time)

When you see a reaction, run through these in order:

Question 1: "Are high-energy bonds being broken or formed?"

This is your **enthalpy antenna**. Certain bond types store a lot of free energy. If the reaction *breaks* a high-energy bond and doesn't form one of comparable energy, it's releasing energy → favors spontaneity. If it *forms* a high-energy bond from lower-energy precursors, that costs energy → nonspontaneous without coupling.

High-energy bond hierarchy (approximate ΔG°' of hydrolysis):

Bond Type	ΔG°' of Hydrolysis	Example
Thioester (C–S)	−31 kJ/mol	Acetyl-CoA
Phosphoanhydride	−30 to −32 kJ/mol	ATP → ADP + Pi
Enol phosphate	−62 kJ/mol	PEP
Acyl phosphate	−43 kJ/mol	1,3-BPG
Glycosidic bond	−15 to −20 kJ/mol	Sucrose, glycogen
Ester bond (ordinary)	~ −10 to −15 kJ/mol	Standard esters
Phosphoester (C–O–P)	−9 to −14 kJ/mol	G6P, F6P

The key insight: Reactions that *hydrolyze* bonds high on this list are spontaneous. Reactions that *form* bonds high on this list from bonds lower on the list are nonspontaneous (unless coupled to something even more favorable).

Question 2: "Does the number of molecules increase or decrease?"

This is your **entropy antenna**.

- **Cleavage reactions** (1 molecule → 2 molecules): entropy increases → favors spontaneity
- **Condensation reactions** (2 molecules → 1 molecule): entropy decreases → opposes spontaneity
- **Decarboxylation** (releases CO_2 as a gas): big entropy gain → strongly favors spontaneity
- **Isomerizations** (same number of molecules, same size): entropy ≈ 0 → near equilibrium unless enthalpy dominates

Question 3: "Is charge or resonance being created or destroyed?"

Products that are **more resonance-stabilized** than reactants lower the energy of the system. Key patterns:

- Carboxylate formation ($–COO^-$): two equivalent resonance structures, very stable
- Phosphate release (Pi): extensive resonance delocalization across multiple oxygens
- Relief of electrostatic repulsion (e.g., separating two adjacent negative charges) → favorable

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Reaction-Type Reference Guide

Here's where the real transferable power comes from. Most biochemical reactions fall into a small number of categories. Learn the thermodynamic *personality* of each type, and you can classify many reactions quickly.

TYPE 1: Hydrolysis Reactions → Almost Always Spontaneous

What it looks like: A bond is cleaved by water. One fragment gets –OH, the other gets –H. The total number of molecules increases.

Why it's spontaneous:

- Bond broken, entropy gained (1 → 2 or more molecules)
- Water is at ~55 M concentration in the cell, so mass action strongly favors hydrolysis
- Products are often more resonance-stabilized than reactants

Confidence level: Very high. Default classification: treat hydrolysis as spontaneous unless the bond being broken is unusually low-energy and the products are destabilized.

Worked example: Sucrose + H_2O → glucose + fructose. Glycosidic bond hydrolysis. 1 molecule becomes 2. Large favorable $\Delta G^{\circ\prime} \approx -29$ kJ/mol. **Spontaneous.**

Other common examples:

- ATP + H_2O → ADP + Pi (spontaneous)
- Protein + H_2O → amino acids (spontaneous)
- Any phosphoester hydrolysis (spontaneous, though the magnitude varies)

TYPE 2: Decarboxylation Reactions → Almost Always Spontaneous

What it looks like: CO_2 is released from a substrate. A C–C bond is broken.

Why it's spontaneous:

- CO_2 is a gas — massive entropy gain, especially under physiological conditions where it can diffuse away
- CO_2 is extraordinarily stable (two C=O double bonds, fully oxidized carbon, linear geometry)
- Product removal by diffusion/buffering makes this essentially irreversible in vivo

Confidence level: Very high *in the decarboxylation direction*. The reverse — carboxylation (fixing CO_2) — is almost always nonspontaneous.

Critical corollary → Carboxylation is nonspontaneous. If you see CO_2 being *added* to a molecule (C–C bond formed with CO_2), it's nonspontaneous without coupling.

Worked example: Pyruvate + CO_2 → Oxaloacetate + H^+. This is a *carboxylation*. You're building a C–C bond and trapping a thermodynamically stable gas molecule. **Nonspontaneous.** (In the cell, pyruvate carboxylase drives this by coupling to ATP hydrolysis and using biotin as a CO_2 carrier.)

TYPE 3: Isomerization / Mutase Reactions → Near Equilibrium

What it looks like: A functional group moves within the same molecule, or the molecule rearranges without gaining or losing atoms. Same molecular formula on both sides.

Why it's near equilibrium:

- No bonds of dramatically different energy are broken vs. formed
- No change in number of molecules → $\Delta S \approx 0$
- No significant change in charge distribution or resonance
- Small $\Delta G^{\circ\prime}$, typically within ±5 kJ/mol

Confidence level: High. If the reaction is just shuffling the same atoms around within one molecule and nothing enters or leaves, it's near equilibrium.

Worked example: Glucose-1-phosphate → Glucose-6-phosphate. The phosphate group migrates from C1 to C6 on the same sugar ring. Same atoms, same charge, same number of molecules. **Near equilibrium.** ($\Delta G^{\circ\prime} \approx -7.3$ kJ/mol — small enough that cellular concentrations can shift it either way.)

Other common examples:

- DHAP ⇌ G3P (triose phosphate isomerase) — near equilibrium
- Glucose-6-phosphate ⇌ Fructose-6-phosphate (phosphoglucose isomerase) — near equilibrium
- 2-phosphoglycerate ⇌ 3-phosphoglycerate — near equilibrium
- Citrate ⇌ isocitrate (aconitase) — near equilibrium

Warning: Not ALL isomerizations are near equilibrium. If the rearrangement creates or destroys a high-energy feature (like converting an ordinary phosphoester into an enol phosphate, as in enolase), the ΔG can be significant. But for simple sugar phosphate isomerizations and mutase reactions, near equilibrium is a useful default classification.

TYPE 4: Aldol Cleavage → Nonspontaneous (Standard), Context-Dependent (Cellular)

What it looks like: A C–C bond in the middle of a sugar or sugar phosphate is broken, producing two smaller carbonyl compounds (typically an aldehyde and a ketone). This is the reverse of an aldol condensation.

Why it's nonspontaneous under standard conditions:

- You ARE gaining entropy (1 → 2 molecules), which helps
- But the C–C bond being broken is a *regular* C–C bond — there's no special driving force to break it
- The enthalpic cost of breaking a C–C bond exceeds the entropic benefit at standard state
- $\Delta G^{\circ\prime}$ for aldolase ≈ +23.8 kJ/mol — substantially positive

Why cellular conditions can flip this:

- In the cell, the products (DHAP and G3P) are continuously removed by downstream enzymes
- G3P is consumed by GAPDH; DHAP is converted to G3P by TPI
- This pulls the equilibrium forward (Le Chatelier)
- Actual cellular ΔG ≈ −1.3 kJ/mol — slightly spontaneous, but barely

Worked example: Fructose-1,6-bisphosphate → DHAP + G3P.

- Under standard conditions: **Nonspontaneous** (ΔG°' is large and positive)
- Under cellular conditions: Classified here as **nonspontaneous**, though many sources report a slightly negative cellular ΔG. The important nuance is that it is *borderline*, while the ΔG°' is emphatically positive.

The general principle: Aldol cleavage (breaking a C–C bond in a sugar to give two carbonyls) has an unfavorable ΔG°'. The reverse — aldol condensation — is thermodynamically favored under standard conditions.

TYPE 5: Phosphoryl Group Transfer / Kinase Reactions → Depends on What's Giving and Receiving

This is the one category where you actually need to *compare* the donor and acceptor.

The rule: Phosphoryl transfer flows spontaneously from compounds with *more negative* ΔG°' of hydrolysis to compounds with *less negative* ΔG°' of hydrolysis.

Think of it like a waterfall: phosphoryl groups "flow downhill" on the phosphoryl transfer potential table.

Phosphoryl Transfer Potential (ΔG°' of hydrolysis, approximate):

Compound	ΔG°' (kJ/mol)
PEP	−62
1,3-BPG	−49
Creatine phosphate	−43
ATP (→ ADP)	−30.5
Glucose-1-phosphate	−21
Glucose-6-phosphate	−14
Glycerol-3-phosphate	−9

If the phosphoryl group moves from a higher compound to a lower one → spontaneous. If it moves from a lower compound to a higher one → nonspontaneous (requires coupling).

Example: PEP + ADP → Pyruvate + ATP. PEP is higher on the table than ATP. Phosphoryl transfer is "downhill." **Spontaneous.**

Example: Glucose + ATP → G6P + ADP. ATP is higher than G6P. Phosphoryl transfer is "downhill." **Spontaneous.**

TYPE 6: Oxidation-Reduction → Follow the electrons to the higher-potential acceptor

The rule: Electrons flow spontaneously from lower reduction potential (more negative $E°'$) to higher reduction potential (more positive $E°'$). A positive $\Delta E°'$ means the reaction is spontaneous (since $\Delta G°' = -nF\Delta E°'$).

Practical shortcut: If NAD^+ or FAD is being *reduced* (gaining electrons from a substrate), it's usually part of an oxidation that is either spontaneous or near equilibrium. If NADH is being *oxidized* (donating electrons to the electron transport chain), it's strongly spontaneous.

TYPE 7: Condensation / Biosynthetic Coupling → Almost Always Nonspontaneous Alone

What it looks like: Two molecules combine into one, often releasing water or another small molecule. C–C bonds, C–N bonds, or C–O bonds are *formed*.

Why it's nonspontaneous:

- Entropy decreases (2 → 1 molecules)
- Building bonds costs energy unless the new bond is lower in energy than what was sacrificed
- Biosynthesis = thermodynamic uphill climb

This is why these reactions are always coupled to ATP hydrolysis, thioester cleavage, or other exergonic processes in the cell.

Examples:

- Amino acid + tRNA → aminoacyl-tRNA (coupled to ATP → AMP + PPi, then PPi → 2Pi)
- Acetyl-CoA + oxaloacetate → citrate (driven by thioester hydrolysis of CoA linkage)
- Pyruvate + CO_2 → oxaloacetate (coupled to ATP in pyruvate carboxylase)

Standard vs. Cellular Conditions: When Does It Matter?

Standard conditions ($\Delta G°'$): All solutes at 1 M, pH 7, 25°C. This is a *reference point*, not reality.

Cellular conditions (ΔG): Actual metabolite concentrations, which are usually far from 1 M (typically micromolar to low millimolar).

The equation connecting them:

$\Delta G = \Delta G^{\circ\prime} + RT \ln Q$

where Q = [products]/[reactants] at actual cellular concentrations.

When the distinction matters:

The only time standard vs. cellular changes the classification is when $\Delta G^{\circ\prime}$ is **moderately positive** (roughly +5 to +30 kJ/mol) AND cellular concentrations strongly favor the forward direction (products kept low, reactants kept high).

Classic examples where the flip occurs:

- **Aldolase:** $\Delta G^{\circ\prime} = +23.8 \rightarrow$ cellular $\Delta G \approx -1.3$ (products drained by downstream enzymes)
- **Triose phosphate isomerase:** $\Delta G^{\circ\prime} = +7.5 \rightarrow$ cellular $\Delta G \approx -2.5$ (G3P consumed rapidly)
- **GAPDH + PGK coupled:** individually variable, but product removal drives forward

If $\Delta G^{\circ\prime}$ is **very negative** (< -15 kJ/mol), cellular conditions won't flip it. It stays spontaneous. If $\Delta G^{\circ\prime}$ is **very positive** ($> +30$ kJ/mol), cellular conditions almost never flip it either. It stays nonspontaneous.

The gray zone — roughly −5 to +25 kJ/mol — is where you need to think about whether the cell is actively managing concentrations.

Quick-Recognition Decision Tree

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Look at the reaction
│
├── Is CO₂ being RELEASED?
│ └── YES → Almost certainly SPONTANEOUS (decarboxylation)
│
├── Is CO₂ being FIXED (added to a molecule)?
│ └── YES → NONSPONTANEOUS (carboxylation)
│
├── Is a bond being HYDROLYZED by water?
│ └── YES → Almost certainly SPONTANEOUS
│ (glycosidic, phosphoanhydride, thioester, peptide, ester)
│
├── Is it an ISOMERIZATION or MUTASE? (same formula both sides)
│ └── YES → Probably NEAR EQUILIBRIUM
│ (unless creating/destroying a high-energy feature)
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│
├── Is it an ALDOL CLEAVAGE? (sugar splits into two carbonyls)
│ └── YES → NONSPONTANEOUS (standard), possibly spontaneous (cellular)
│
├── Is it a CONDENSATION? (two molecules → one, building a bond)
│ └── YES → Probably NONSPONTANEOUS unless coupled
│
├── Is it a PHOSPHORYL TRANSFER?
│ └── Compare donor and acceptor on the phosphoryl transfer table
│ High → Low = SPONTANEOUS
│ Low → High = NONSPONTANEOUS
│
└── Is it a REDOX reaction?
└── Electrons flowing to higher reduction potential = SPONTANEOUS
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Worked Examples for independent study

Reaction 1: Pyruvate + CO_2 → Oxaloacetate + H^+

Identify the type: Carboxylation (CO_2 is being fixed, not released).

Apply the rule: Carboxylation = building a C–C bond with thermodynamically stable CO_2. Entropy decreases (2 reactants → fewer degrees of freedom). No high-energy bond is being broken to pay for it.

Answer: Nonspontaneous. ✓

In the cell, pyruvate carboxylase couples this to ATP → ADP + Pi and uses a biotin cofactor.

Reaction 2: Fructose-1,6-bisphosphate → DHAP + G3P (Standard Conditions)

Identify the type: Aldol cleavage. A C3–C4 bond in a hexose bisphosphate is cleaved to give two triose phosphates.

Apply the rule: C–C bond cleavage costs energy. Even though entropy increases (1 → 2), the enthalpic cost dominates. $\Delta G^{\circ\prime}$ = +23.8 kJ/mol.

Answer: Nonspontaneous. ✓

Reaction 3: Same Reaction Under Cellular Conditions

Apply the cellular correction: Products are consumed rapidly. But ΔG°' is quite positive (+23.8). Whether the actual cellular ΔG is slightly negative or slightly positive depends on exact concentrations. It's in the gray zone — borderline.

Working classification: Nonspontaneous. (Some sources say slightly spontaneous under cellular conditions. A conservative classification: if the ΔG°' is this positive, lean nonspontaneous unless you have strong reason to believe product removal is extreme.)

Reaction 4: Glucose-1-phosphate → Glucose-6-phosphate

Identify the type: Isomerization (phosphate migration within the same sugar).

Apply the rule: Same molecular formula, same charge, same number of molecules. Nothing dramatic changes. ΔG°' ≈ −7.3 kJ/mol — small magnitude.

Answer: Near equilibrium. ✓

Reaction 5: Sucrose + H_2O → Glucose + Fructose

Identify the type: Hydrolysis (glycosidic bond cleavage by water).

Apply the rule: Hydrolysis of a glycosidic bond. 1 molecule → 2 molecules. Entropy gain. Products (free monosaccharides) are stable. ΔG°' ≈ −29 kJ/mol.

Answer: Spontaneous. ✓

Common reasoning pitfalls and How to Avoid Them

Trap 1: "More molecules = spontaneous." Not always. Entropy helps, but if you're breaking a strong bond to get those extra molecules without a thermodynamic payoff (like aldol cleavage), the enthalpy cost can dominate. Entropy alone doesn't guarantee spontaneity.

Trap 2: Confusing the direction. Decarboxylation is spontaneous; carboxylation is not. Hydrolysis is spontaneous; condensation is not. Always identify which direction the reaction is going.

Trap 3: Forgetting cellular vs. standard. Under explicitly stated cellular conditions, consider whether Le Chatelier shifts the equilibrium. Reactions with moderate positive ΔG°' can be flipped by product removal. Reactions with large positive ΔG°' generally cannot.

Trap 4: Assuming all phosphoryl transfers are spontaneous because "ATP hydrolysis is favorable." The deciding factor is where the phosphoryl group is going: ATP donating to glucose (forming G6P) is downhill. But ATP donating to PEP (which has higher transfer potential) would be uphill — that direction doesn't happen.

Trap 5: Treating ΔG°' and ΔG as interchangeable. ΔG°' is a fixed property of the reaction at standard state. ΔG is what actually determines spontaneity in the cell. They can have opposite signs.

Summary: The Five Thermodynamic Instincts

1. **Hydrolysis and decarboxylation are thermodynamically downhill.** These are the cell's "money moves." When you see water cleaving a bond or CO_2 flying off, think spontaneous.
2. **Condensation and carboxylation are thermodynamically uphill.** Building bonds and fixing CO_2 cost energy. These always need coupling.
3. **Isomerizations and mutase reactions sit near equilibrium.** Same stuff on both sides, small ΔG, easily reversible.
4. **Aldol cleavage is enthalpically uphill but entropically helped.** At standard state, enthalpy wins → nonspontaneous. Under cellular conditions, it can go either way depending on product removal.
5. **Phosphoryl transfer follows a potential table.** High-potential donors spontaneously phosphorylate low-potential acceptors. The table is your map.

Internalize these five instincts and you'll be able to analyze virtually any biochemical spontaneity pattern without memorizing individual ΔG values.