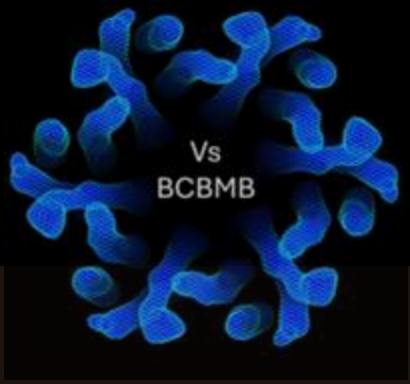
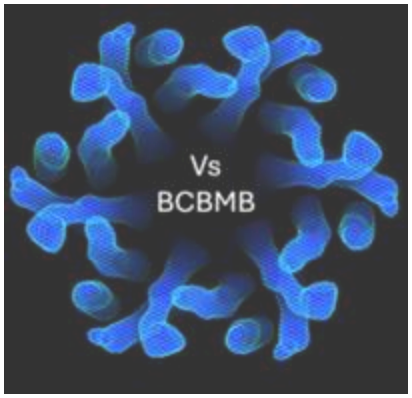


PART 2

How Do Molecules "See"?



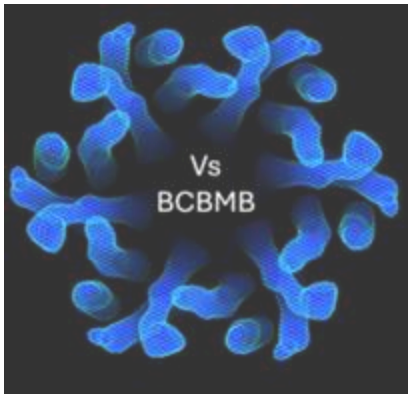
RECAP – Part 1

Weak Interactions Allow Molecules to "See" - Why?

Summary Conclusion: The **combination of energy** (=strength) **and geometry** (=spatial distribution) is perfect to give molecules "vision" because it **allows to substitute sight with scanning (=process) of the physical and chemical surface properties** that result from the strategic placement/display of functional groups by the scaffold.

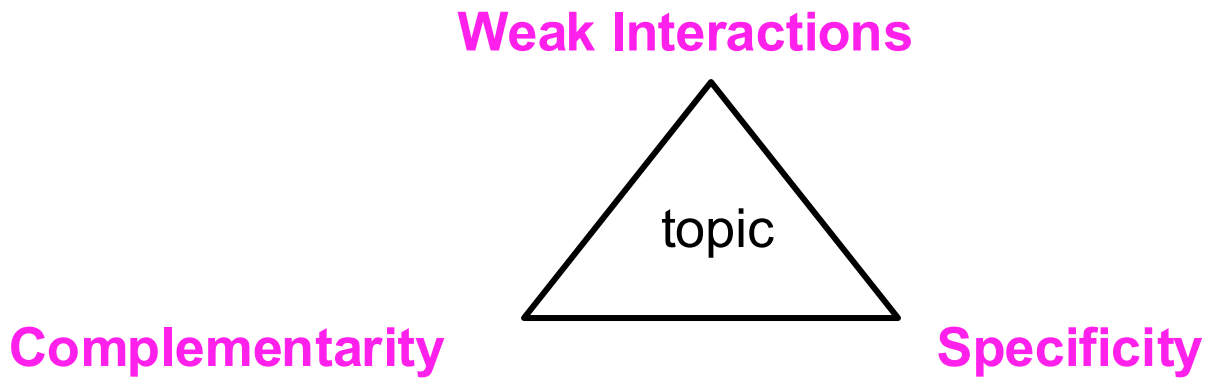
➔ Molecular sight amounts to a **pattern recognition** process; the short lifetime of weak interactions allows this scanning to happen at the timescale of molecular collisions (covered in a different lecture that explains how and why enzymes function at all)

➔ **molecular recognition and specific engagement occur when two surfaces are mutually matched through complementary physical and chemical properties**

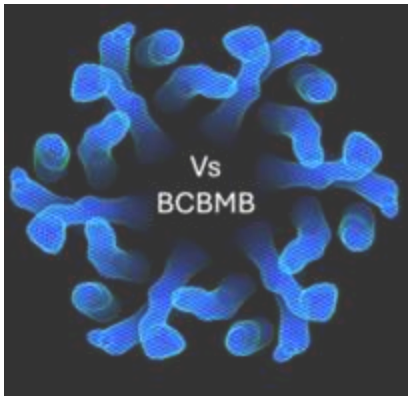


RECAP - Part 1

The following diagram arguably is the **singly most important correlation for understanding ALL of life at the molecular level.**

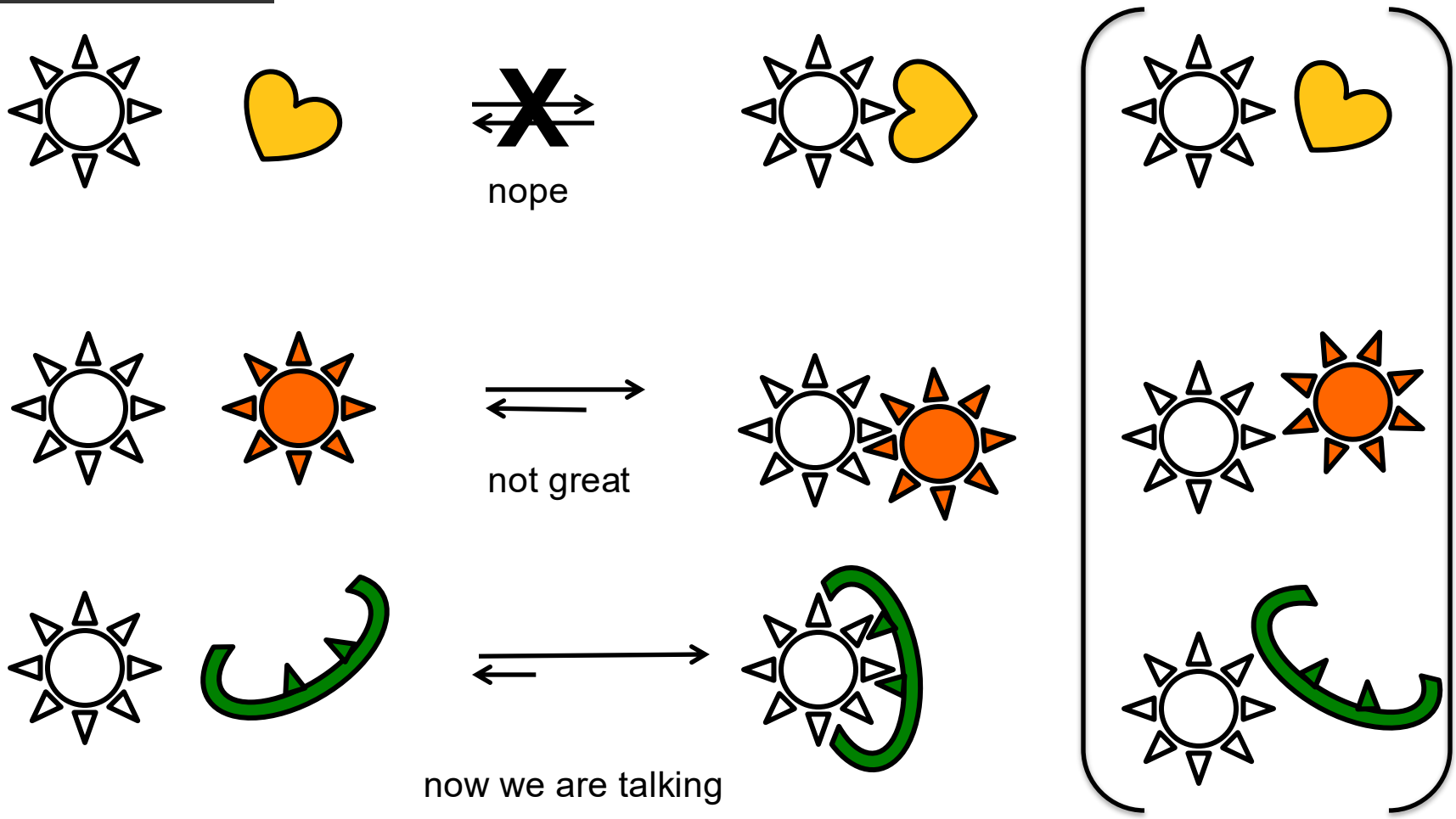


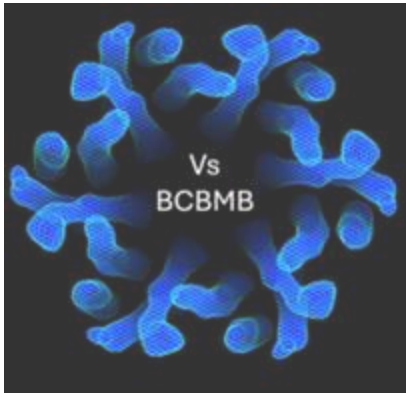
The significance of this diagram lies in its simple message: **if it doesn't fit, it doesn't interact.** (eg a pair of glasses and your knee).



From “Seeing” to “Selecting”

An almost offensively simplified way of representing the “lay summary” could look like this.





Lets put this idea of “fit” in context of Orgo you (hopefully) **know....**

What does “sp^3” stand for?

→ an atom (often carbon) with tetrahedral geometry of the substituents

What “real world“ object do you associate with this?

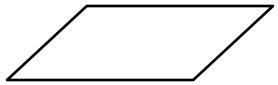
→ "pyramid", tetrahedron

What does “sp^2” stand for?

→ an atom (often carbon) with planar geometry of the substituents

What “real world“ object do you associate with this?

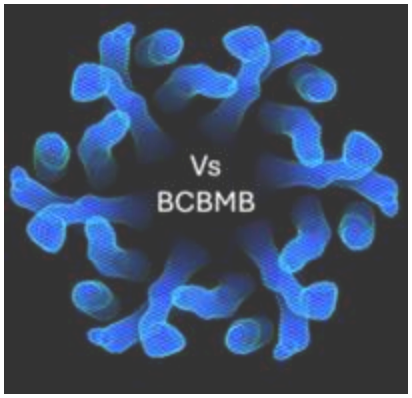
→ sheet of paper



What has that got to do with “fit” and recognition?

→ Different use of space

→ Different requirements for “fit” and recognition

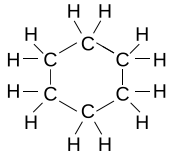


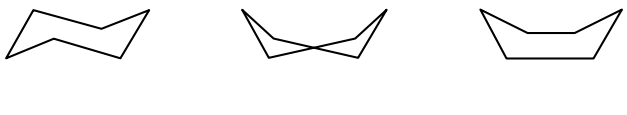
Molecular Recognition – Basic Understanding

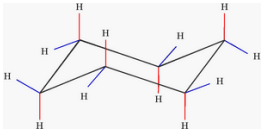
We will look at just two molecules here, but this time we will focus on **spatial** issues

Cyclohexane (sp^3)

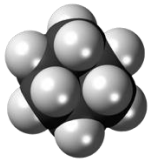
top view







side view(s)



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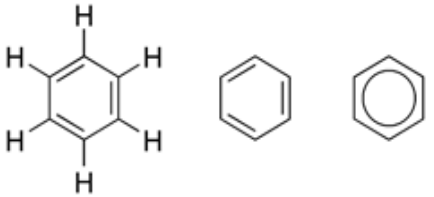
Benzene (sp^2)



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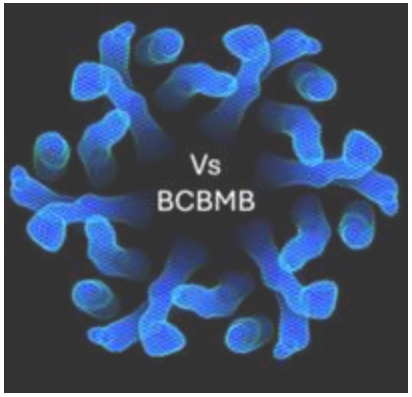
https://upload.wikimedia.org/wikipedia/commons/6/67/Benzene_structure.png

which one is easier to “understand”?

→ based on what we learned so far: the one that requires less extensive “touching”

→ =benzene - hands down - because “understanding” the structure and properties of cyclohexane requires exploration in 3D (substituents, conformational diversity), while “understanding” benzene can be restricted to exploring the perimeter. (incidentally, this is **really** important for understanding how/why nucleic acids work….)



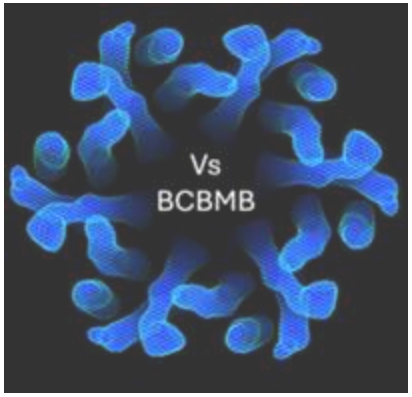
From “Seeing” to “Selecting”

- How molecules “see” = recognize each other (Pt1)
- **How do molecules select other molecules to engage with?**
- Whether these engagements will be a “one night stand” or more serious, and
- What implication molecular recognition mechanisms have for molecular evolution.

→ This comes down to appreciating the difference between “acknowledging” and “engaging” (in human terms).
→ To start this: lets look at our “lay summary” again... **if it doesn’t fit, it doesn’t interact.**

Question: when you encounter a stranger on an otherwise deserted sidewalk – what do you do?

→ (if you don't ignore the person) you probably say “hi” or “nod”
→ but: do you stop to have a conversation?
→ In all likelihood: no → why?
→ Likely answer: because you don’t know that person, and you are not interested to get to know them.
→ How would you get to know them?
→ Through more extended interactions that tell you whether you “like” this person/want to interact more/again
→ How does that whole dynamic change if you already DO know the person?
→ You may stop and have a (quick) chat or not (if you’re in a hurry)
→ **HOW** is this **RELEVANT** for understanding “mechanisms of molecular selectivity” **??**



From “Seeing” to “Selecting”

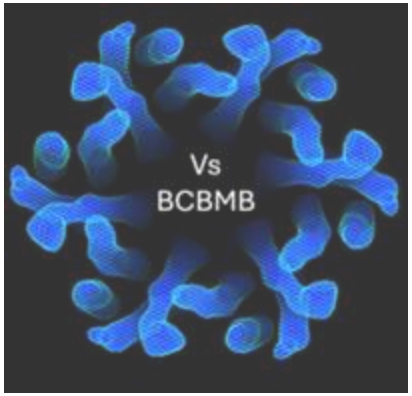
Here is why the “real life” example is relevant (I think):

→ in all likelihood you say “hi” or “nod”
This is an “acknowledgement”. Molecules constantly acknowledge each other because their diffusion and motion within a very crowded environment causes trillions of molecular encounters/collisions every second.

In each collision: chemical groups/probes encounter each other AND engage in weak interactions, BUT

→ a single, non-covalent weak interaction is easily broken by thermal energy. (just like saying “hi” most often does not lead to a more extensive contact)

→ **formation of a single interaction does not suffice to stably associate two molecules.**



From “Seeing” to “Selecting”

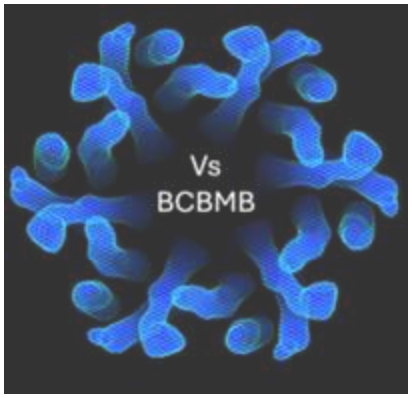
Here is why the “real life” example is relevant (I think):

Putting this In words that acknowledge thermodynamics: two molecules in the cell interact stably (and truly see each other) when the **overall change** in Gibbs Free Energy of their pairwise interaction is negative (= spontaneous association) **and** larger than the thermal energy that tries to disrupt things by giving molecules kinetic energy.

$$\Delta G < 0$$

G: Gibbs Free Energy

How does that help us understand molecular recognition?



From “Seeing” to “Selecting”

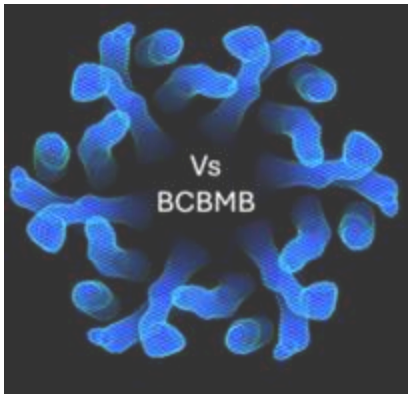
Lets take a closer look at Gibbs Free Energy Change:

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

G: Gibbs Free Energy
H: Enthalpy (measure of heat exchange)
T: Temperature
S: Entropy (measure of disorder)

At first sight this may be confusing. Why?

Weak interactions reduce molecular motion of the **participating atoms** (“bad”), but in most cases also release heat (“good”). The net outcome (and hence the sign of ΔG) is difficult/impossible to determine for just a single interaction because it depends on too many other things (eg solvent effects, or entropy changes in other parts of the molecule [think of the effect tickling may have on you]).



From “Seeing” to “Selecting”

Lets take a closer look at Gibbs Free Energy Change:

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

G: Gibbs Free Energy
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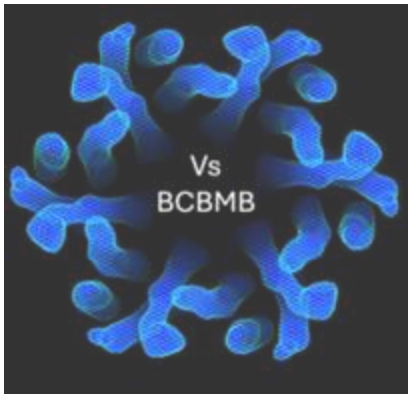
T: Temperature
S: Entropy (measure of disorder)

.....however….if two molecules are complementary to each other = can form **multiple** simultaneous interactions, then the net release of heat (negative ΔH) and any cumulative gain in entropy for the entire system (eg free up water) outweighs the local loss in entropy of interacting groups (negative ΔS) resulting in:

$$\Delta G_{overall} < 0$$

….favoring the constructive interaction of the molecular entities.

This only leaves us with the task to put the meaning of the above into some sort of quantitative and visual form. Doing so brings us to another **hugely** important concept…..



From “Seeing” to “Selecting”

Molecular scale “seeing/selecting/associating”

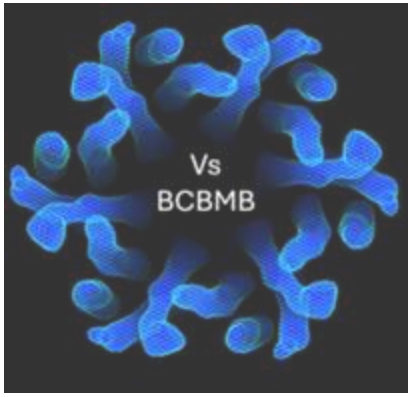
summarily referred to as

“binding”

is based on

equilibrium

= binding is <u>NOT</u> a static process



From "Seeing" to "Selecting"

"binding" – is based on _____
(= it is _____)

Picking two arbitrary molecules, A and B, this means

$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AB$$

k_{on}: on rate; k_{off}: off rate

$$K_a = \frac{[AB]}{[A][B]} = \frac{k_{on}}{k_{off}}$$

Association constant

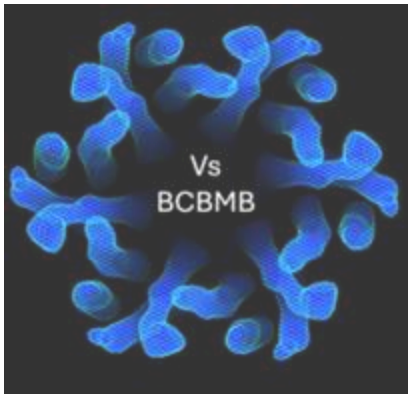
or: more commonly used in biochemistry

$$K_d = \frac{1}{K_a} = \frac{k_{off}}{k_{on}}$$

Dissociation constant

Looking at this, you notice: molecular interactions depend on the [concentration] of the participants

→ This makes sense because [ ] affects the frequency of collisions. Increasing either [A] or [B] will shift the binding equilibrium towards AB.



From “Seeing” to “Selecting”

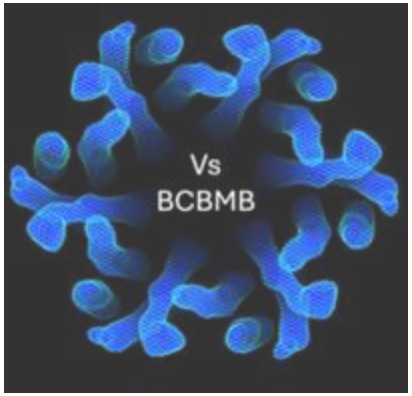
$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AB$$

k_{on}: on rate; k_{off}: off rate

Moreover: …. interactions will be more favorable if the “on-rate” is large, and the “off-rate” is small (= you capture but let go slowly), leaving us with the question……

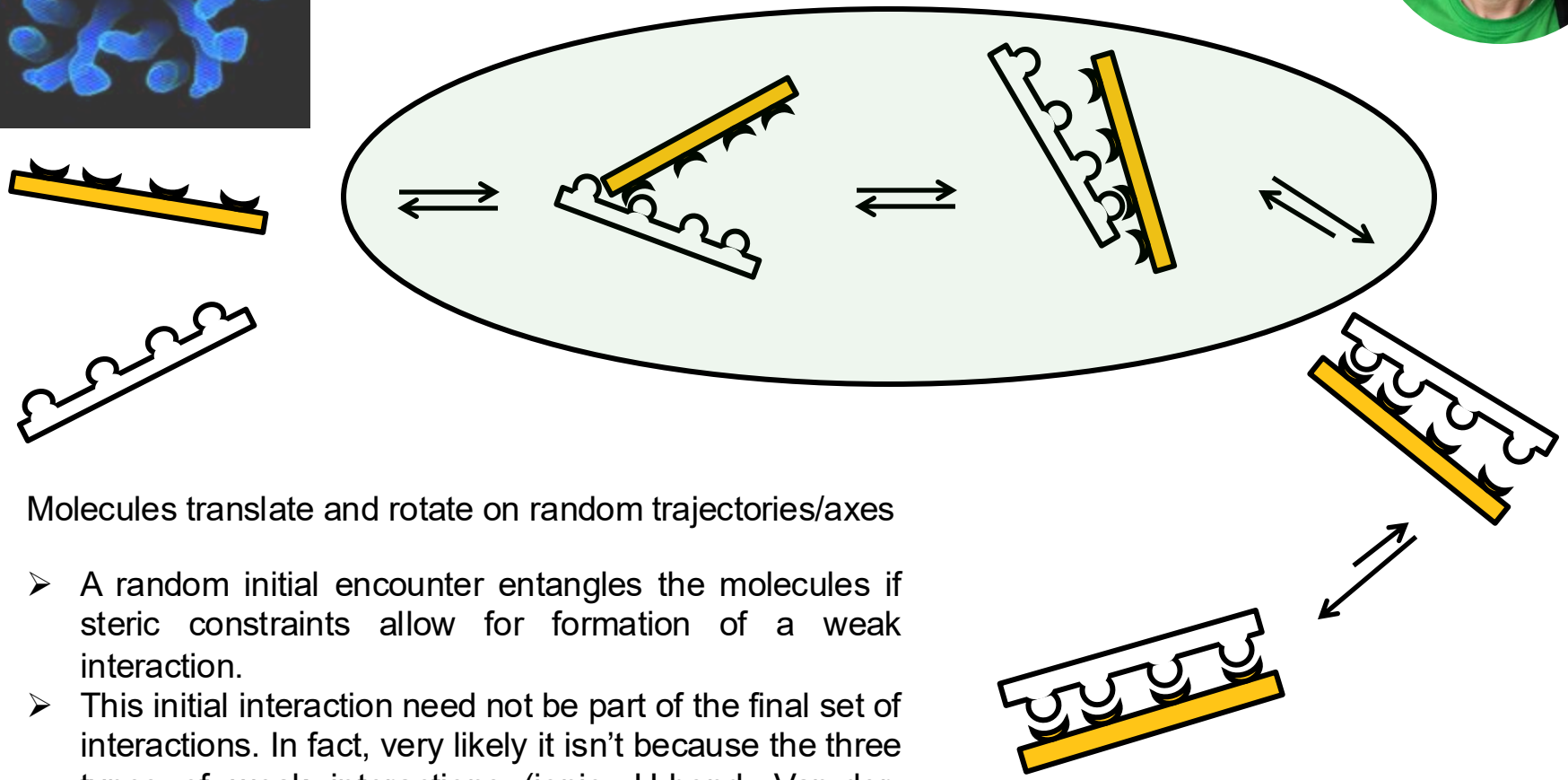
What determines the magnitude of “on-/off-rates”?

→ Here it becomes critical to remember that a singular weak interaction is **not** sufficient to cause stable molecular associations. → so, lets look at what happens if you have multiple weak interactions.....



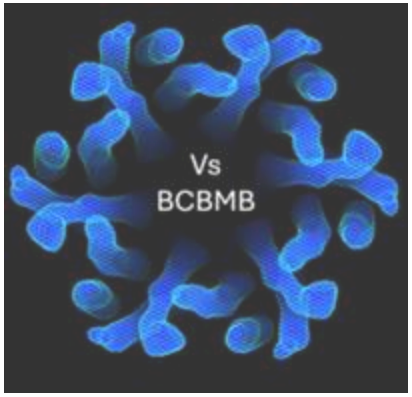
From “Seeing” to “Selecting”

The schematic shows two molecules (orange and white) that have four mutually matched sites that can engage in reciprocal weak interactions.



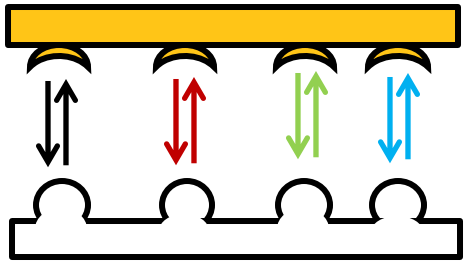
Molecules translate and rotate on random trajectories/axes

- A random initial encounter entangles the molecules if steric constraints allow for formation of a weak interaction.
- This initial interaction need not be part of the final set of interactions. In fact, very likely it isn’t because the three types of weak interactions (ionic, H-bond, Van-der-Waals), are quite generic.
- However, a more persistent/bound state is sequentially reached **if a better alignment with more interactions is reached before Brownian motion macroscopically separates the molecules.**



From “Seeing” to “Selecting”

As important as the sequential aspect of “binding”:
each of the (degenerate) weak interactions is subject to an equilibrium <u>on its own</u>



Implications:

(1) each of the interactions can be undone

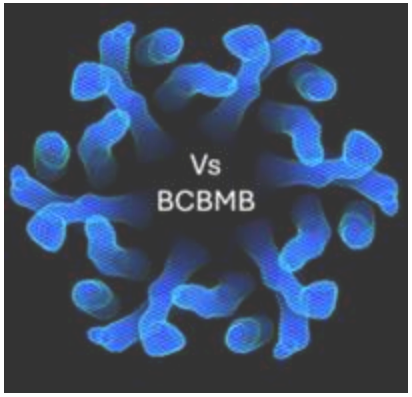
(2) equilibria are coupled to each other

- initial contact between two molecules is very weak with a high likelihood to fall apart
- **BUT:** Interactions that are present at any point increase the likelihood for another interaction to form if complementarity exists, and vice versa: every interaction that is lost increases the likelihood for another interaction to break (a case of “glass half full or half empty”).

In other words: binding **<u>and</u>** dissociation are competing processes that are fully reversible, and cooperative.

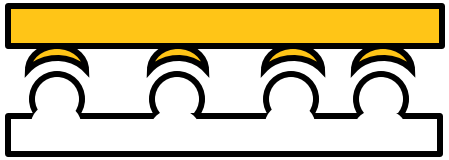
Consequently, **macroscopic “on-/off-rates” represent the ensemble average** of the underlying microstates (… = statistical thermodynamics!)

meaning: number of molecular complexes in which ALL possible interactions are formed is very small; in fact: if you were to take a snapshot of 100 copies of a macromolecular complex at a timescale faster than forming/breaking weak interactions, then there is a good likelihood that each of them will have a different pattern of weak interactions from amongst those that are possible.



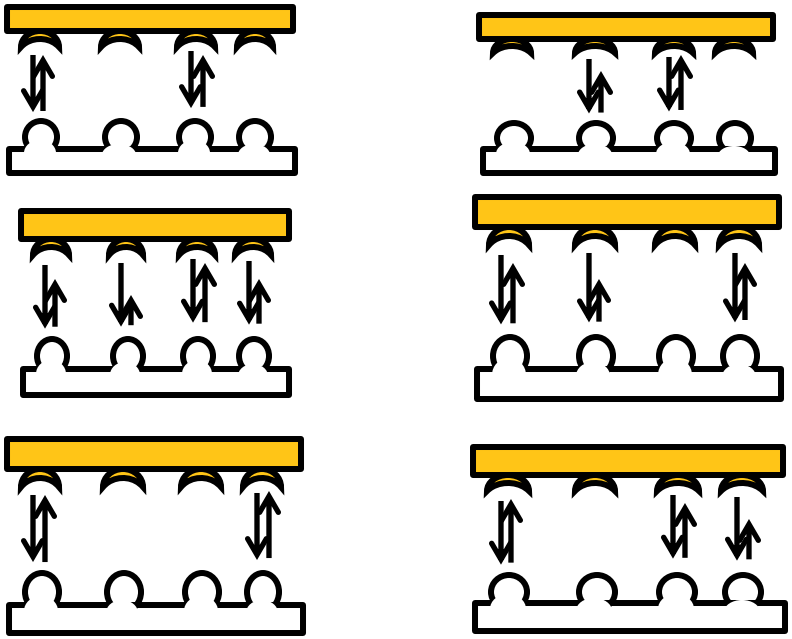
From “Seeing” to “Selecting”

Trying to illustrate the idea of “microstates” gives but one glimpse (and insight) into the randomness of life at the molecular level:

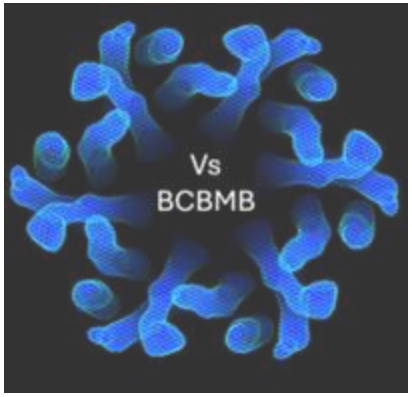


Fortuitously: the **cell is a damped system** (as we discovered in the opening lecture) = at a practical level **cells do not care about <u>individual</u> microstates as long as the ensemble averages are doing what is needed**:

(a) ignore everything that isn’t relevant,
(b) be able to interact when needed,
(c) disassemble when the job is done.



Note: differences in the length of arrows for "on-/off-rates" simply indicate that these microscopic rates are all different in each state. Their actual strength depends on the conformational states of interacting partners, chemical environment, temperature, type of weak interaction, type of neighboring interactions etc ….



From “Seeing” to “Selecting”

Trying to illustrate the idea of “microstates” gives but one glimpse (and insight) into the randomness of life:

While introducing the idea of microstates in molecular interactions may be confusing to you at first, making an effort to think about them **will** help you to **understand** many things in biology and biochemistry (eg: how and why enzymes work, or how processes like transcription, translation and replication are regulated).

I understand that all this may give you a "headache" and if it all gets too much, just try to remember that molecular interactions are very dynamic at the small scales of local environments → this dynamic turns **each and every molecular assembly into a miniature "information processing device" that constantly integrates and responds to ALL incoming cues**…. which is, frankly, pretty amazing.

As a peace offer: for "business as usual", use of **macroscopic** “on-/off-rates” as proxies to evaluate macromolecular interactions is legitimate/sensible because they represent ensemble averages and are directly linked to the Gibbs Free Energy:

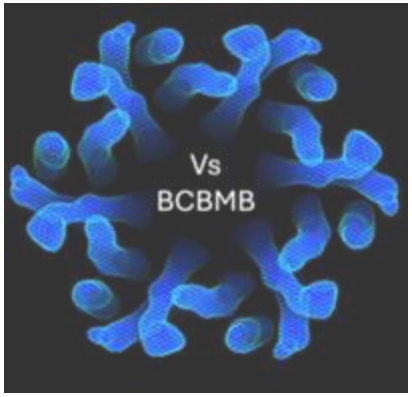
$$\Delta G = RT \ln K_d = RT \ln \frac{k_{off}}{k_{on}}$$

$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AB$$

Where spontaneous binding/selection occurs if $\Delta G < 0$

k_{on}: on rate; k_{off}: off rate

(just keep in mind that sometimes you want to consider the impact that "not behaving like the average" has on everything….it will make your life much easier).



"Selecting" - "Does it Matter?"

- How molecules "see" = recognize each other
- How molecules select other molecules to engage with
- Whether these engagements will be a "shortlived" or more serious
- What implication molecular recognition mechanisms have for molecular evolution.

Shortlived or More Serious?

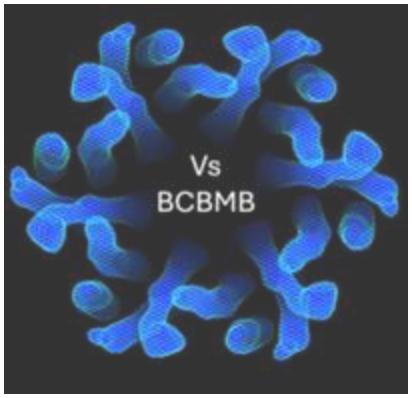
answer here seems quite simple: # and nature of interactions. (the more the merrier)
→ ***in vitro*** that actually **holds true**
→ ***In vivo***, however, things **can be a (very) different story**

Why would it matter whether you are in a test tube or inside a cell? Aren't the relevant interactions the same?

Answer: probably yes – for the most part they are, BUT remember

$$K_d = \frac{1}{K_a} = \frac{k_{off}}{k_{on}} = \frac{[A][B]}{[AB]}$$

I know: "math" doesn't speak to (many of) you (and I confess: it's not my favorite thing to look at or use either)….but please … give it a look and try to see where this is going..... – I know that you can figure this out!



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Answer: probably yes – for the most part they are, BUT remember

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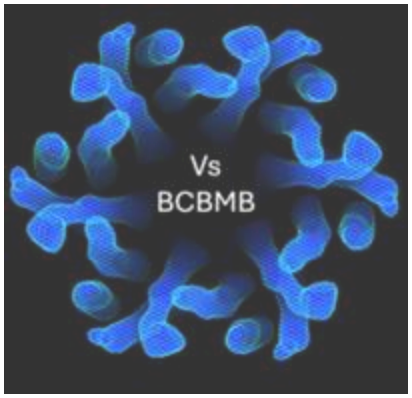
That is: binding and dissociation (assembly/disassembly) are concentration dependent (think: Law of Mass Action).

→ Gives cells means to tinker with molecular interactions by **dynamically manipulating** the concentrations

- synthesize more, degrade
- chemically change to enhance/destroy interactions
- physically sequester or enrich components in the "right/wrong" location
- create spatial distribution that allow one interaction but not others

→ **NONE** of these things can (easily) be replicated *in vitro.* **Hence, be wary of *in vitro* "binding affinities" or "dissociation constants" – they may tell you something physiologically relevant or not ....it really depends....**and as time goes by, cell biologists and biophysicists alike are slowly becoming more sensitized to the fact that **"cellular reality matters".**



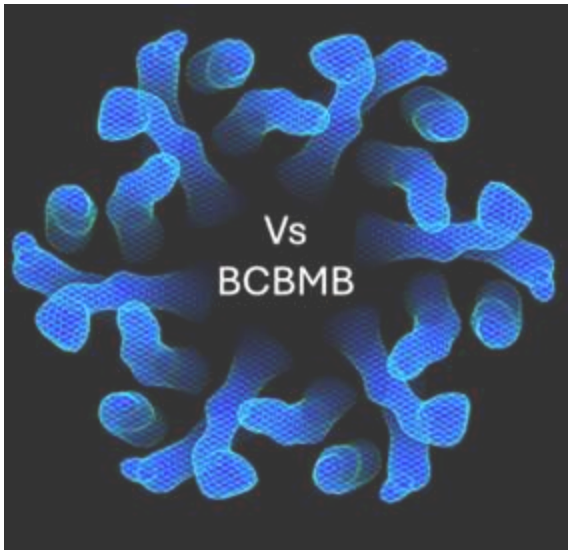
Molecular Recognition Mechanisms and the Emergence of Life

- How molecules "see" = recognize each other
- How molecules select other molecules to engage with
- Whether these engagements will be "shortlived" or more serious
- **What are the implications of molecular recognition mechanisms for molecular evolution?**

Coming back to the structure of the Universe one more time –
very first moments after the "big bang": matter was completely evenly distributed. However, random fluctuations in the local density of matter set in motion the formation of gravity wells….and spontaneous formation of stars, galaxies, and dark matter filaments followed. **In its progression this was a unidirectional process because whatever happened next was a response to what already was.**

Contemplating the emergence of life-
also relied on the self-assembly of molecules that were capable of engaging in non-random interactions. Many of these molecules emerged through random chemistry in the "violent" early atmosphere of Earth, some arrived with asteroids. ➔ Evolution of life was a "unidirectional" process = starting from a few non-random interactions, the next iteration/improvement was built "around" the status quo.

However, **in contrast to the Universe, life figured out how to copy itself by learning how to manipulate spatial properties "at will" and at scales that match the organism they occur in.** That is a truly awe inspiring emergent property, and will be the focus of what we are going to study, now that we have some fundamental understanding for how molecules "see".



Slides are freely available at
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