The Interview: Hemoglobin *vs.* Myoglobin

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SETTING

The setting for this play is the corporate offices of the company Physio Logy. The company is interviewing candidates for key positions in its oxygen transport group.

CAST

HEMO GLOBIN .... Job Candidate #1
MYO GLOBIN...................... Job Candidate #2
PLAS MA............................... Recruiting Manager for Physio Logy
MRS. RED............................ Division Chief of the Oxygen Transport Group
MR. WHITE .... Member of the Interview Panel
PLATE LETS .... Member of the Interview Panel
MR. MUSCLES..................... Deputy Chief and Head, Oxygen and Exercise Branch

SCENE 1

PLAS MA: Hello everybody. Today we are going to be shortlisting candidates to join our team at Physio Logy, the company that has revolutionized the science of bodily functions. The candidate we are looking for should have a very strong work ethic, be available 24–7, have the capacity to carry oxygen in the human body and deliver it in a timely fashion when needed. He or she should also be able to carry a heavy load of carbon dioxide and dispose of it according to waste disposal regulations and be willing to work with human resources in regards to salary and fringe benefits. So is everybody clear on the specific requirements of the job?

PLATE LETS and *MR. WHITE:* Yes, absolutely!

MRS. RED: Plas Ma, I have something else to add to the requirement since I will be working very closely with the new hire.

PLAS MA: Sure, go ahead and tell us what's on your mind.

MRS. RED: Well, I strongly believe that the candidate should be a team player and willing to forgo any kind of vacation time.

PLAS MA: Does everyone agree with Mrs. Red?

PLATE LETS: Yes I do, and we should add the requirements to the job description.

MR. WHITE: Seconded!

PLAS MA: Okay then, I will go ahead and add it to the job requirements. Now I think it is time to review the candidates for the job. Mrs. Red, do you have your shortlist?

(Mrs. Red ruffles through her papers to find the shortlisted candidates.)

MRS. RED: So, we have shortlisted four candidates for the job: Hemo Cyanin, Hemo Globin, Hemo Erythrin and Myo Globin.

PLAS MA: Well, I do think that judging from their previous work experience we can rule out Hemo Cyanin and also Hemo Erythrin. Hemo Cyanin and Hemo Erythrin only seem to have previous experience in invertebrates. So I propose that we call Hemo Globin and Myo Globin for the interview. Are we all agreed?

EVERYONE (together): Yes!

∽ SCENE 2 ∽

A few days later both the candidates arrive at the offices of Physio Logy for the job interview. First up is Hemo Globin. Hemo Globin strides confidently into the interview room.

PLAS MA: Good morning Mr. Hemo Globin, and how are you today? Did you have any difficulty finding our offices? Please have a seat.

HEMO GLOBIN (replying confidently): I am very well, thank you for asking. No, I didn't have any problems finding your offices. I'm one of those molecules that isn't afraid to ask for directions.

PLAS MA: Good to hear, Mr. Hemo Globin. I'm Plas Ma the hiring manager, and my job within the company is to make sure that the oxygen transport and bodily defense teams function smoothly. I will let my team leaders introduce themselves to you.

MRS. RED: Good morning, Mr. Hemo Globin, nice to meet you. I am Red Blood Cell (RBC), Mrs. Red for short. If you are hired for the position, then you will be working very closely with me in the Oxygen Transport Department.

HEMO GLOBIN: It's entirely my pleasure, Ma'am. I very much look forward to the opportunity.

(Mrs. Red blushes and fans herself with her papers.)

PLATE LETS: Hello, I'm Plate Lets and my expertise mainly lies in defense and repair, but I work very closely with the other members of the team to help stop bleeding and the loss of other team members.

HEMO GLOBIN: Nice to meet you, Mr. Plate Lets.

MR. WHITE: Good morning, Mr. Hemo Globin. I'm White Blood Cell (WBC); you can call me Mr. White. I am trained extensively in defensive functions. My team and I protect the entire organization from unexpected attacks by extra-cellular terrorists.

HEMO GLOBIN: Very nice to meet you, Mr. White. You have such an honorable job.

MR. WHITE: Thank you, Mr. Hemo Globin, very kind of you to say so!

PLAS MA: Now that we have the pleasantries taken care of, let's get down to business. Mr. Hemo Globin, please tell us a little bit about yourself to start off with.

HEMO GLOBIN: I really don't like to talk about myself, but since you asked, I will try and give you a sense of who I am and what I do. I am a protein made up of chains of amino acids folded to form a globular structure with a hydrophobic (water-hating) core and a hydrophilic (water-loving) exterior. I can carry oxygen and carbon dioxide and in some cases nitric oxide.

PLATE LETS: Mr. Hemo Globin, can you tell me a few more details about your structure please?

HEMO GLOBIN: My pleasure, Mr. Plate Lets. I happen to have four polypeptide chains made up of amino acids joined together by peptide bonds. My chains are named alpha and beta. I have two alpha and two beta chains. My primary structure *[Figure 1a]* can further fold up to give rise to secondary structure elements, such as alpha helices and loops. Most proteins also have beta sheets but I am comprised primarily of alpha helices.

MRS. RED: What exactly is an alpha helix and how is it different from a beta sheet?

HEMO GLOBIN: Good question! An alpha helix is a right-handed spiral structure that is formed due to twisting of the polypeptide chain. There are on average 3.6 amino acid residues per turn of the helix; it looks like a corkscrew. My alpha helical structure is stabilized by the formation of hydrogen bonds between the amino acids making up the chain *[Figure 1b]*. On the other hand beta sheets are made up of amino acid chains folded up to form a fan-like structure *[Figure 1b]* stabilized by inter-strand hydrogen bonding. Both these elements are repetitive structures and occur many times in a protein molecule. The alpha helices and beta sheets are joined together by loops or random coils to form a tertiary structure.

MRS. RED: Thank you Mr. Hemo Globin, that was very helpful.

MR. WHITE: Yes, yes this is all very well but what does this have to do with you carrying out your job?

PLATE LETS: Now, now, Mr. White, let's not get impatient. Let's give Mr. Hemo Globin a chance to explain himself.

HEMO GLOBIN: Thank you, Mr. Plate Lets. I was just getting to that point, but first I need to explain my quaternary structure to everyone. As I was saying, alpha helices and beta sheets connected by random coils come together to form a tertiary structure *[Figure 1c]* per chain. As I mentioned, I have four chains—two alpha chains and two beta chains. When all four of my chains come

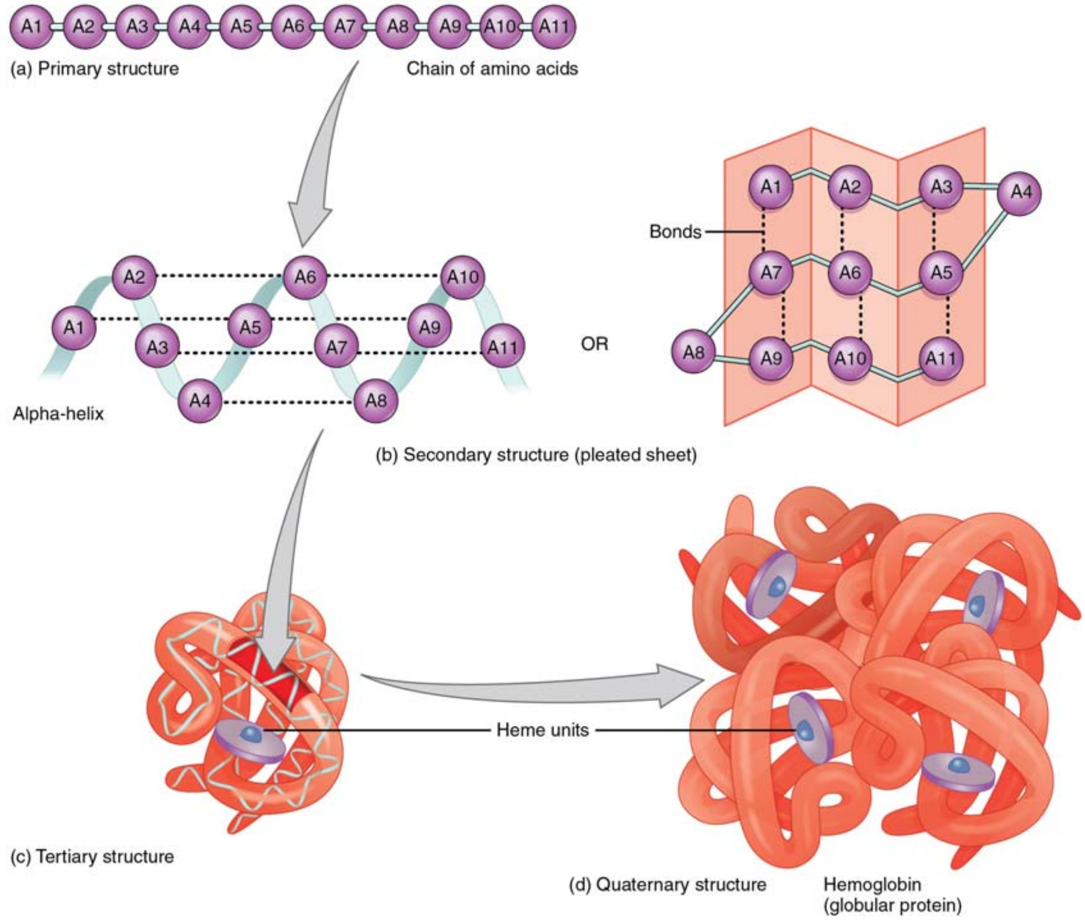

Figure 1. The primary, secondary, tertiary and quaternary structures of hemoglobin.
Source: OpenStax College, http://commons.wikimedia.org/wiki/File:225_Peptide_Bond-01.jpg, CC BY 3.0.

together and are coordinated with heme (iron and the porphyrin ring) in each chain, this gives rise to my quaternary structure *[Figure 1d]*. Each chain is called a "subunit," and when all four of my subunits come together then I am complete *[Figure 2].* Because I have four subunits I am referred to as a tetrameric protein, and because I contain iron I am also referred to as a metallo protein. My quaternary structure thus contains more than one polypeptide chain that differentiates it from the tertiary structure. Just in case you are wondering, my iron ion can bind oxygen and the porphyrin ring surrounding my iron ion is responsible for binding globin *[Figure 3]*.

MRS. RED: This is absolutely fascinating, what an extremely complicated structure you have!

HEMO GLOBIN: Why thank you Mrs. Red, I will take that as a compliment! Yes, my structure is rather complicated and it was solved way back in 1959 by a scientist named Max Perutz, who co-shared the Nobel prize in 1962 for elucidating my structure.

MR. WHITE (intrigued): Hmmm, how exactly did Max Perutz discover your structure?

HEMO GLOBIN: I'm glad you asked that question, Mr. White. He used a technique called X-ray crystallography. He first of all used various chemical solutions to make me form crystals. Once he had nice, well-

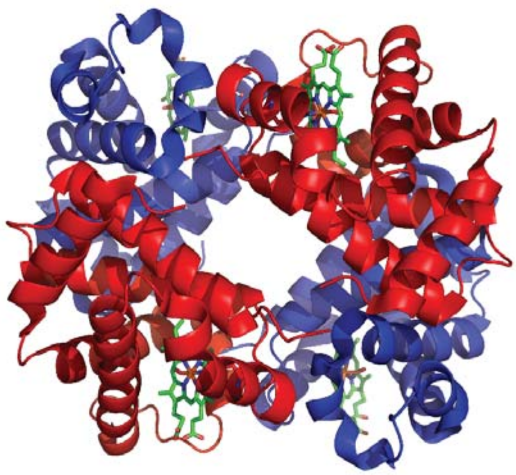

Figure 2. Three-dimensional structure of hemoglobin.
Source: Richard Wheeler, http://commons.wikimedia.org/wiki/File:1GZX_Haemoglobin.png, CC BY-SA 3.0.

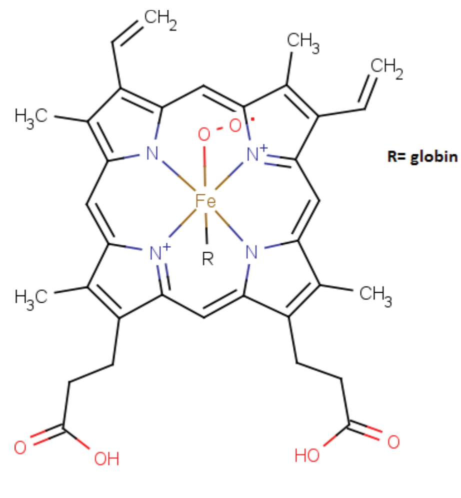

Figure 3. Oxygen binding to hemoglobin.
Source: Gladissk, http://commons.wikimedia.org/wiki/File:Structures_of_Hemoglobin_forms.png, CC BY-SA 3.0

formed crystals, he passed X-rays through me (a process called X-Ray diffraction). The crystals scattered (diffracted) the X-rays and the points of diffraction formed a pattern on an X-ray film that was placed behind me. Using this pattern he could make complicated calculations to determine my structure.

MR. WHITE: This is fascinating stuff, but how does your structure make you well suited for the job?

HEMO GLOBIN: I was just coming to that part, sir. The component of my structure responsible for binding oxygen is the iron/heme *[Figure 3]*. Since I have four subunits, each with its own iron, I can bind up to four molecules of oxygen at a time.

PLAS MA: How does this compare with Mr. Myo Globin?

HEMO GLOBIN (very smugly): My understanding is that he can carry only one molecule of oxygen at a time!

MR. WHITE: Is that so? Hmmm…

(Mr. White writes furiously in his notebook.)

HEMO GLOBIN: To the best of my knowledge, sir, it appears to be true.

PLATE LETS: So where exactly does oxygen binding occur?

HEMO GLOBIN: In the arterial blood. Because I'm oxygenated, the arterial blood appears a brighter shade of red due to oxygen binding to my red iron groups.

MRS. RED: So it is you who imparts color to the red blood cells?

HEMO GLOBIN: Yes ma'am, I believe so!

MRS. RED: Can you explain how exactly the oxygen binds to your heme?

HEMO GLOBIN: Absolutely! The fact of the matter is that oxygen is not very soluble in an aqueous solution such as blood so it needs to bind to me in order to be transported. Each of my iron ions can bind one oxygen molecule so I can carry four oxygen molecules at a time. This is called my oxygen carrying capacity. The reason why oxygen binding to me is very efficient is because of a process called "cooperative" binding.

PLAS MA: So what exactly is cooperative binding?

HEMO GLOBIN: Very simply, it means that once one oxygen molecule has bound to me I can change shape (undergo a conformational change) rapidly in order for the 2nd, 3rd, and 4th molecules of oxygen to bind to me more efficiently. Changes in the structure of one of my subunits are translated into structural changes in adjacent subunits. My structure can change with the binding of oxygen from a low affinity "T" (taut) state to a high affinity "R" (relaxed) state.

PLATE LETS: How does that work?

HEMO GLOBIN: When my iron is oxygenated it moves closer to the porphyrin ring. As a result, the histidine residue (to which the iron atom is attached) is drawn closer to the heme group. This movement of the histidine residue then shifts the position of other amino acids that are near it and the structure of the interface (where the four subunits interact) is altered, which leads to other structural changes. What this means is that when a single heme group in me becomes oxygenated, my entire protein changes its shape (allostery). In my new shape, it is easier for the other three heme groups to become oxygenated. Thus, the binding of one molecule of oxygen enhances my ability to bind more oxygen molecules. This property is known as "cooperative binding" *[Figure 4]*.

I can bind oxygen cooperatively so that in places of the body with high oxygen concentrations (such as in the lung) I can take up oxygen. The cooperative binding of oxygen enables me to deliver almost 1.7 times as much oxygen than it would if the sites were independent. My

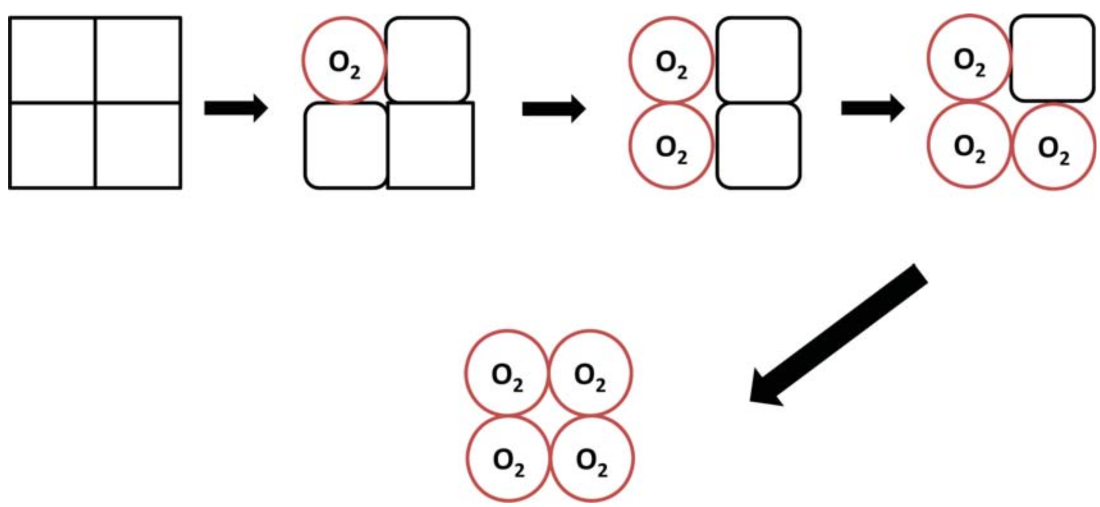

Figure 4. Cooperative binding of oxygen to hemoglobin. The binding of one oxygen molecule causes a change in conformation allowing the 2nd, 3rd and 4th oxygen molecules to bind more efficiently to hemoglobin. Source: Karobi Moitra, own work.

regulation by oxygen dramatically increases my physiological oxygen-carrying capacity. In the tissues where oxygen concentration is low I can give up oxygen also in a cooperative manner, thus releasing oxygen efficiently into the tissues.

MR. WHITE: Okay, I get that you can efficiently carry oxygen, but how do you release oxygen, carry carbon dioxide and release it into the lungs for disposal?

HEMO GLOBIN: Excellent question! This can be explained by a phenomenon called the Bohr effect where hydrogen ions and carbon dioxide help to promote the release of oxygen.

MRS. RED: It all sounds very complicated to me!

HEMO GLOBIN: Not at all, Ma'am. It's actually very simple. My oxygen affinity decreases as pH decreases from the value of 7.4 (pH decrease means that the hydrogen ion concentration becomes higher). Thus, as I move into regions of low pH, I tend to release oxygen. In addition, I respond to carbon dioxide with a decrease in oxygen affinity, which helps the release of oxygen in tissues with a high carbon dioxide concentration. This type of regulation of oxygen binding by hydrogen ions and carbon dioxide is called the Bohr effect after Christian Bohr, who described this phenomenon in 1904. I am carried with bound carbon dioxide and hydrogen ions in the blood back to the lungs. In the lungs I release the hydrogen ions and carbon dioxide and rebind oxygen. Thus, I help to transport hydrogen ions and carbon dioxide in addition to transporting oxygen.

PLAS MA: Mr. Hemo Globin, thank you very much for your time. The conversation with you has been extremely interesting. We will let you know the results of the interview at a later date.

HEMO GLOBIN: Thank you all for having me. I look forward to working with you all in the future.

(Hemo Globin exits the room confidently.)

PLAS MA: Well, ladies and gentlemen, please fill up your evaluations and I will call in the next candidate.

(Plas Ma switches on the intercom and instructs his secretary to call Myo Globin into the interview.)

SCENE 3

(Enter Myo Globin.)

PLAS MA: Mr. Myo Globin, I'm Plas Ma. I am very happy to meet you. Please take a seat.

MYO GLOBIN: Thank you so much.

(Myo Globin takes a seat.)

PLAS MA: Mr. Myo Globin, let me introduce you to Mrs. Red, Mr. White and Plate Lets.

MYO GLOBIN: Pleased to meet you all.

MRS. RED: Mr. Myo Globin, I'm the Division Chief for the Oxygen Transport Group. Can you tell me in a few words why you would be a suitable candidate for our team?

MYO GLOBIN: Certainly Mrs. Red. I am a globular monomeric protein found primarily in the muscles and I can bind oxygen molecules delivered by Hemo Globin. I am capable of binding a single oxygen molecule.

MRS. RED: I see; so you are not primarily found in the blood?

MYO GLOBIN: No Ma'am.

(Mrs. Red writes busily in her notebook.)

MRS. RED: Thank you. Can you tell me a little bit about your structure?

MYO GLOBIN: I contain a single polypeptide chain and am built primarily of alpha helices and random turns. I contain a heme group (made up of a protoporphyrin and a central iron atom) that allows me to reversibly bind oxygen. My interior consists almost entirely of hydrophobic (water-hating) residues. The only charged residues that I have inside me are the two histidines that play a vital role in binding of oxygen.

PLATE LETS: So what is the mechanism by which you can bind oxygen?

MYO GLOBIN: It involves a bit of complicated chemistry. My iron can bind to four nitrogen atoms in the center of the protoporphyrin ring, leaving two free bonding sites. One site binds to the histidine, the other is free to bond with oxygen. This histidine prevents other larger molecules from entering the oxygen-binding site and also makes the reaction reversible.

PLATE LETS: So what you are saying is that the oxygen carrying capacity is one molecule of oxygen per molecule of Myo Globin?

MYO GLOBIN: Yes, that is correct.

MR. WHITE: How does your structure differ from that of Mr. Hemo Globin?

MYO GLOBIN: Hemo Globin is a tetramer composed of two each of subunits alpha and beta. I'm a monomer, so I don't have a quaternary structure at all *[Figure 5]*.

PLAS MA: So in terms of function how do you differ from Mr. Hemo Globin?

MYO GLOBIN: Well sir, I can bind oxygen much more tightly than does Hemo Globin. That's why I am well suited for binding oxygen

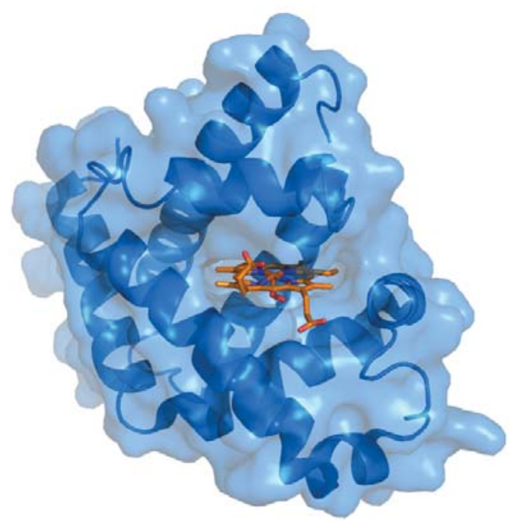

Figure 5. Cartoon representation of myoglobin (blue) with heme group (orange). *Source:* Thomas Splettstoesser, http://commons.wikimedia.org/wiki/File:Myoglobin_and_heme.png, CC BY-SA 3.0.

in the muscle. Additionally, I can act as a back-up system for Hemo Globin under extreme conditions like exertion or exercise. When Hemo Globin cannot supply enough oxygen to the muscle I can take over because I am able to store oxygen. Since I hold onto oxygen more tightly, letting go of it isn't easy; as a result I am able to store oxygen reserves in the muscles.

PLAS MA: So what you are basically saying is that you don't release oxygen into the blood; but why?

MYO GLOBIN: I don't release oxygen into the blood because I have a higher affinity for oxygen than Hemo Globin. Furthermore, the partial pressure of oxygen in mitochondria is much lower than blood so that when I do give up my oxygen, the concentration gradient favors delivery to mitochondria rather than blood. This property can be illustrated with the help of graphs depicting my higher affinity for oxygen compared to Hemo Globin *[Figure 6]*.

MRS. RED: Oh, now I understand; so you would be more useful in the environment of the skeletal muscle when Hemo Globin is under excessive pressure?

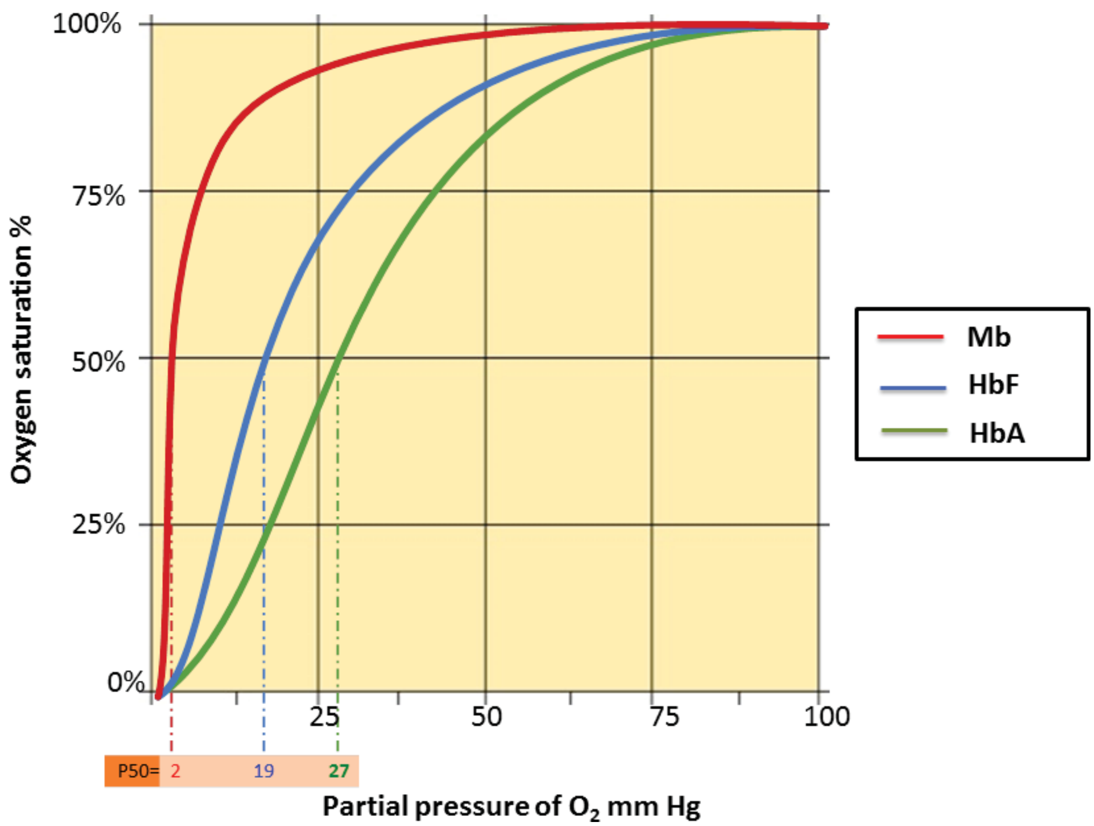

Figure 6. Graphical representation of oxygen saturation curves of myoglobin, fetal hemoglobin and hemoglobin A (Mb-myglobin, HbF- fetal hemoglobin, HbA- hemoglobin A).

The hemoglobin (HbA) curve is S-shaped (sigmoidal) in character signifying the change in oxygen affinity over a small range of oxygen concentration allowing hemoglobin to almost fully unload oxygen at the tissues. Myoglobin (Mb), on the other hand, must effectively bind any oxygen released from hemoglobin (HbA). Myoglobin therefore needs to have a higher oxygen affinity than hemoglobin as illustrated by the hyperbolic curve.

Source: Figure modified by Karobi Moitra from Wikimedia commons, original author Michal Komorniczak, http://commons.wikimedia.org/wiki/File:Saturation_HbA-HbF-Mb.svg, CC BY 3.0.

MYO GLOBIN: That's correct, Ma'am. We like to work as a team, Hemo Globin and I. My buddy Hemo Globin carries the oxygen from the bloodstream, then passes some of it to me in the muscle cells for storage until it is needed in stressful situations such as exercise.

MRS. RED: So as I mentioned, I'm the Division Chief for the Oxygen Transport Group; however, my deputy Mr. Muscles is responsible for recruitment to the Oxygen and Exercise Branch of the division. Let me see if he is available to join us for the interview.

MYO GLOBIN: That sounds like a good idea.

(Mrs. Red calls Mr. Muscles and asks him to join the group for discussion. Mr. Muscles walks in and takes a seat.)

MRS. RED: Mr. Muscles, do you have any specific questions for the candidate since this is your area of expertise?

MR. MUSCLES: Absolutely! Thank you Mrs. Red for calling me. Now Mr. Myo Globin, it is my understanding that you function primarily in the skeletal muscles rather than in the blood. Why is this?

MYO GLOBIN: Well, as I was explaining before, the blood needs to be able to lose oxygen as well as capture it. The whole point is to take oxygen into the lungs and then release it throughout the body. Blood that is really good at only capturing oxygen would be an oxygen trap. If I were to be present in the blood I would not want to let go of the oxygen because I have a high affinity for it. That is why I take oxygen from hemoglobin and store it in the muscle for future use.

MR. MUSCLES: Good answer!

PLATE LETS: I have one last question for Mr. Myo Globin. Why do deep-diving sea mammals have higher concentrations of Myo Globin?

MYO GLOBIN: Deep-diving sea mammals take long underwater dives. In order to have enough stored oxygen to allow them to do this, they need a much higher concentration of me. Usually when proteins are highly concentrated in one place, they start to clump together, which stops them from functioning properly. In the case of deep diving sea mammals, they have evolved variants of Myo Globin that are less likely to clump together because they have a highly charged surface that allows them to remain at high concentrations without a loss of function.

PLAS MA: Good answer. Thank you for your time, Mr. Myo Globin. We will let you know our decision at a future date.

MYO GLOBIN: Thank you very much everyone, I look forward to hearing from you.

(Myo Globin exits the room gracefully.)

∽ SCENE 4 ∽

PLAS MA: Now ladies and gentlemen, we have the very difficult task of choosing whom to hire for the job: Mr. Hemo Globin or Mr. Myo Globin?

MRS. RED: It's not difficult at all, Mr. Plas Ma, I choose Hemo Globin for my oxygen transport division. He is more valuable to me than Myo Globin.

MR. MUSCLES: But Mrs. Red, my branch could not function properly without Mr. Myo Globin!

PLATE LETS and MR. WHITE (together): So whom do we hire?

(PLAS MA looks down into his notebook, ruffles his papers and concentrates intently for a few seconds then speaks.)

PLAS MA: We hire them both! Mr. Hemo Globin will take care of oxygen transport in the blood, while Mr. Myo Globin will take care of oxygen storage and release in the muscle. Does that sound reasonable to everyone?

EVERYONE (together): Yes! Great decision!

PLAS MA: Okay then, let's get all the paperwork done and invite both Mr. Hemo Globin and Mr. Myo Globin to join the Physio Logy team.

(Cheers erupt all around the room.)

Questions

1. Describe the structure of hemoglobin. How is the structure of hemoglobin suitable for oxygen transport?
2. What is cooperative binding? Explain the cooperative binding of oxygen to hemoglobin.
3. How many molecules of oxygen can one molecule of hemoglobin bind? Why?
4. How many molecules can one molecule of myoglobin bind? Why is this number different from that of hemoglobin? Why do you think that myoglobin binds oxygen more tightly than hemoglobin?
5. Highlight the difference between an alpha helix and a beta sheet. Which kind of secondary structural element are both myoglobin and hemoglobin primarily composed of?
6. Which component of both myoglobin and hemoglobin is required for binding oxygen. Briefly describe the mechanism of oxygen binding in myoglobin.
7. Explain the Bohr effect.
8. Explain why hemoglobin is more suitable for carrying oxygen in the blood and myoglobin more adept at storing oxygen in the skeletal muscles.
9. Prepare a resume and cover letter for either myoglobin or hemoglobin highlighting their particular skill set for the job.
10. Research the roles of secondary structural elements (alpha helices and beta sheets) in proteins. Explain some of the functional roles of these elements in proteins.

Reference

Berg, J. M., J. L. Tymoczko, and L. Stryer. 2002. *Biochemistry*. 5th ed. New York, WH Freeman. Sections 3.8 and 10.2.