Investigating the Structural Basis of HMG-CoA Reductase Cofactor Specificity

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Abstract

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGR) catalyzes the committed and rate-limiting step of the mevalonate pathway of isoprenoid precursor biosynthesis.[1] To optimize isoprenoid production, greater structural and mechanistic understanding of HMGR is necessary.

This study aims to examine the structural basis of cofactor specificity of class II HMGR using homologs from *Enterococcus faecalis* (EfHMGR), an NADPH-preferring HMGR, and *Bordetella petrii* (BpHMGR), an NADH-preferring HMGR, through X-ray crystallography. In addition, the genomes of 15 HMGR-expressing organisms were analyzed to determine the relationship between HMGR cofactor preference and the isoprene synthesis pathways encoded by the genome.

Background

The mevalonate (MEV) pathway synthesizes the building blocks of isoprenoids, the largest and most structurally diverse class of natural products, many of which are used as drugs for the treatment of human diseases.[2] Catalyzing the committed and rate-limiting step of the mevalonate pathway, HMGR reduces HMG-CoA to mevalonate using two equivalents of NAD(P)H **(Figure 1)**.[1]
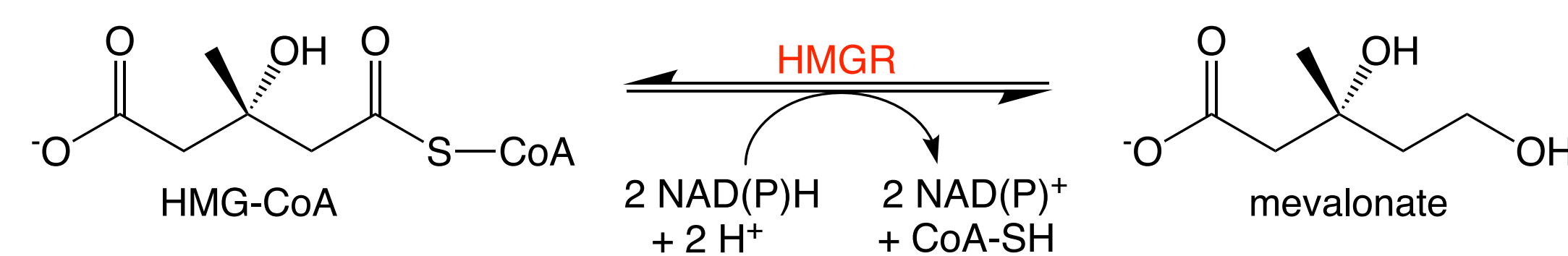

Figure 1. HMGR reduces HMG-CoA to mevalonate using NADH or NADPH.

Two classes of HMGR have evolved: class I HMGRs, which are found in eukaryotes and in some bacteria and archaea, utilize NADPH exclusively (Figure 2), while class II HMGRs, which are found in bacteria and archaea, vary in cofactor preference for either NADH or NADPH (Figure 3).[3-4]
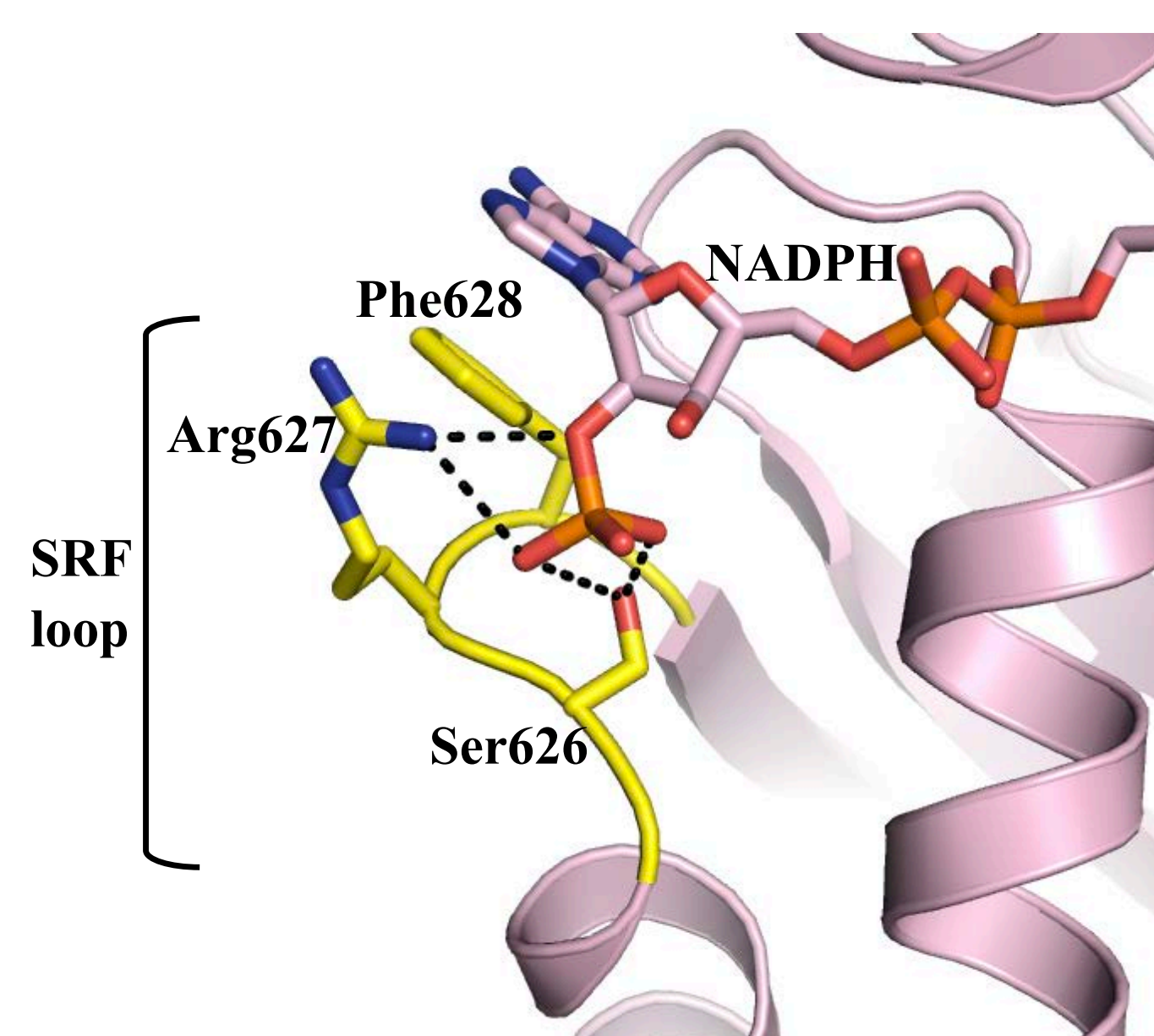

Figure 2. Structure of NADPH-bound human class I HMGR, with SRF loop in yellow.
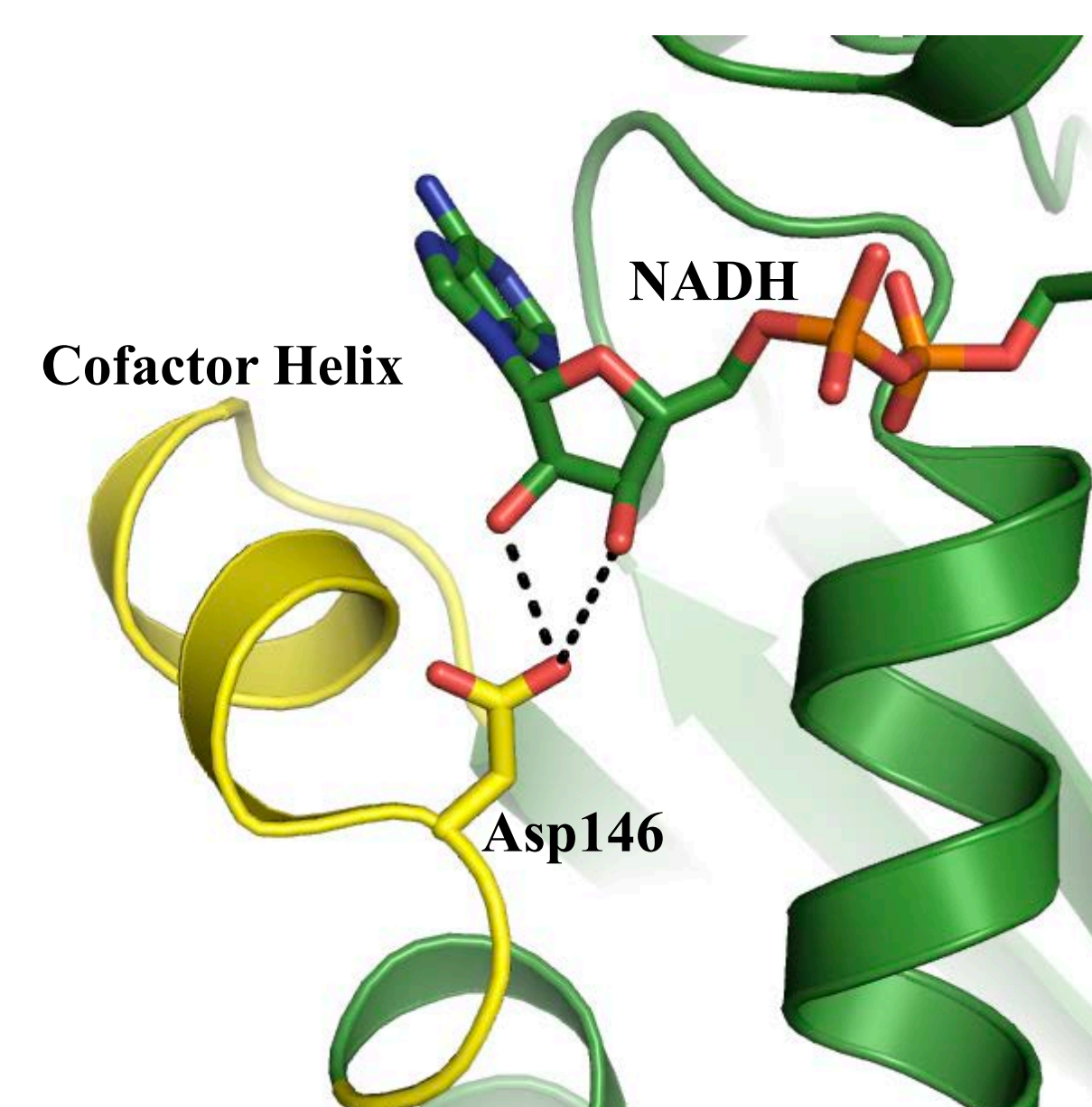
Figure 3. Structure of NADH-bound class II HMGR from *Pseudomonas mevalonii* (PmHMGR), with cofactor helix in yellow.

HMGR Cofactor Specificity and Isoprene Biosynthesis Pathways

Organism	HMGR Class	HMGR Cofactor Preference	Enzymes of MEV Pathway											
			acetoacetyl-CoA thiolase	3-hydroxy-3-methylglutaryl-CoA synthase	3-hydroxy-3-methylglutaryl-CoA reductase	mevalonate kinase	phosphomevalonate kinase	mevalonate phosphate decarboxylase	mevalonate pyrophosphate decarboxylase	isopentenyl phosphate kinase	isopentenyl pyrophosphate isomerase Type 1	isopentenyl pyrophosphate isomerase Type 2
Haloferax volcanii (Archaea, Euryarchaeota)	Class I	NADPH[5]	√	√	√	√		√		√		√	
Sulfolobus solfataricus (Archaea, Crenarchaeota)	Class I	NADPH[6]	√	√	√	√	√	√		√	√		√
Homo sapiens (Eukarya)	Class I	NADPH	√	√	√	√			√		√	√	
Mesocricetus auratus (Eukarya)	Class I	NADPH	√	√	√	√			√		√		
Saccharomyces cerevisiae (Eukarya)	Class I	NADPH	√	√	√	√			√		√		
Listeria monocytogenes (Bacteria, Eubacteria)	Class II	NADPH[4]	√	√	√	√			√				√
Enterococcus faecalis (Bacteria, Firmicutes)	Class II	NADPH[7]	√	√	√	√			√				√
Staphylococcus aureus (Bacteria, Firmicutes)	Class II	NADPH[4]	√	√	√	√			√				√
Streptococcus pneumoniae (Bacteria, Firmicutes)	Class II	NADPH[4]	√	√	√	√			√				√
Borrelia burgdorferi (Bacteria, Spirochaetes)	Class II	NADPH[8]	√	√	√	√			√				√
Archaeoglobus fulgidus (Archaea, Euryarchaeota)	Class II	NADH[4]	√	√	√			√		√		√	
Methanocaldococcus jannaschii (Archaea, Euryarchaeota)	Class II	NADH[9]	√	√	√			√		√			
Bordetella petrii (Bacteria, Proteobacteria)	Class II	NADH[7]	√		√								
Burkholderia cenocepacia (Bacteria, Proteobacteria)	Class II	NADH[4]	√	√	√				√		√		
Delftia acidovorans (Bacteria, Proteobacteria)	Class II	NADH[10]	√		√								

Figure 4. MEV pathway enzymes present in representative archaea, bacteria, and eukaryotes. Checkmarks indicate enzymes identified in the genome. Sequences were obtained using BLASTP.

Organism	HMGR Class	HMGR Cofactor Preference	Enzymes of DXP Pathway										
			1-deoxy-D-xylulose-5-phosphate synthase	1-deoxy-D-xylulose-5-phosphate reductoisomerase	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	4-Hydroxy-3-methylbut-2-enyl diphosphate reductase
Haloferax volcanii (Archaea, Euryarchaeota)	Class I	NADPH[5]							
Sulfolobus solfataricus (Archaea, Crenarchaeota)	Class I	NADPH[6]							
Homo sapiens (Eukarya)	Class I	NADPH							
Mesocricetus auratus (Eukarya)	Class I	NADPH							
Saccharomyces cerevisiae (Eukarya)	Class I	NADPH							
Listeria monocytogenes (Bacteria, Eubacteria)	Class II	NADPH[4]	√	√	√	√	√	√	√
Enterococcus faecalis (Bacteria, Firmicutes)	Class II	NADPH[7]				√	√		
Staphylococcus aureus (Bacteria, Firmicutes)	Class II	NADPH[4]	√	√	√	√	√	√	√
Streptococcus pneumoniae (Bacteria, Firmicutes)	Class II	NADPH[4]	√	√	√	√	√	√	√
Borrelia burgdorferi (Bacteria, Spirochaetes)	Class II	NADPH[8]							
Archaeoglobus fulgidus (Archaea, Euryarchaeota)	Class II	NADH[4]							
Methanocaldococcus jannaschii (Archaea, Euryarchaeota)	Class II	NADH[9]							
Bordetella petrii (Bacteria, Proteobacteria)	Class II	NADH[7]	√	√			√		√
Burkholderia cenocepacia (Bacteria, Proteobacteria)	Class II	NADH[4]	√	√		√	√	√	√
Delftia acidovorans (Bacteria, Proteobacteria)	Class II	NADH[10]	√	√		√	√	√	√

Figure 5. DXP pathway enzymes present in representative archaea, bacteria, and eukaryotes. Checkmarks indicate enzymes identified in the genome. Sequences were obtained using BLASTP.
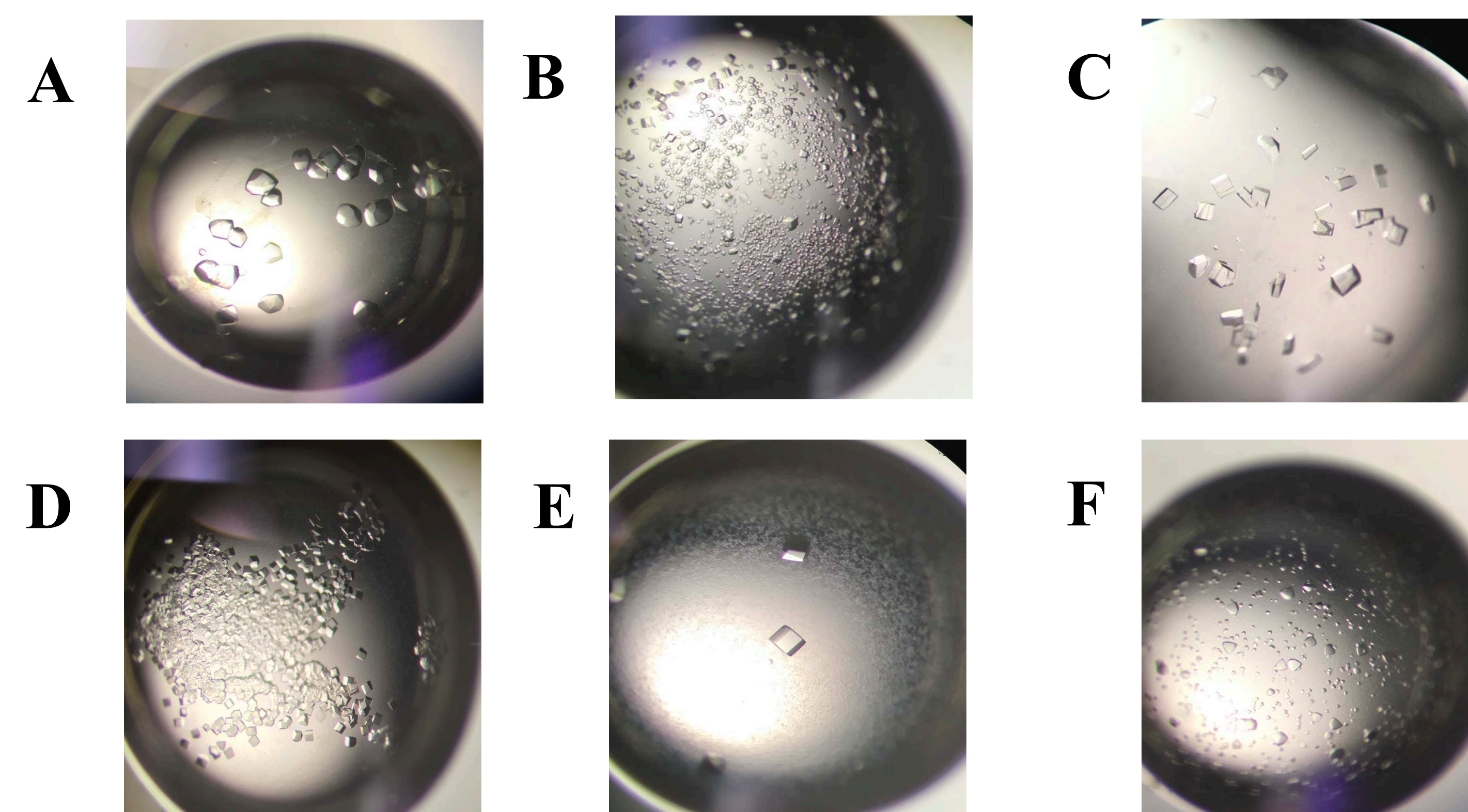
Protein Crystallization

Figure 6. BpHMGR crystals. Crystallization conditions:
(A)–(C) 18%-23% polyethylene glycol (PEG) 3350, 0.2 M ammonium sulfate, 0.1 M HEPES pH 7.5, with 5 mM mevalonate, and 1 mM NADH.
(D) 24% PEG 3350, 0.3 M lithium sulfate, 0.1 M HEPES pH 7.5, with 5 mM mevalonate and 1 mM NADH.
(E) 23% PEG 3350, 0.2 M potassium citrate, with 1 mM NADH.
(F) 1.16 M ammonium sulfate, 0.1 M HEPES pH 7.5, with 1 mM NADH.

Future Directions

❖ Crystallization conditions will be optimized for EfHMGR and BpHMGR.
❖ Additional sequence alignment studies will be performed to examine metabolic pathways of additional organisms.

Acknowledgement

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