Simultaneous solving high-resolution structures of various enzymes from human kidney microsomes

Meinan Lyu, Chih-Chia Su, Masura Miyagi, and Edward Yu
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Corresponding author(s): Edward Yu, Case Western Reserve University

Review Timeline:

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
Dear Dr. Yu,

Thank you for submitting your manuscript entitled "Simultaneous determination of high-resolution structures of a variety of enzymes from human kidney microsomes" to Life Science Alliance. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revised manuscript addressing the Reviewer comments.

To upload the revised version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. Please note that papers are generally considered through only one revision cycle, so strong support from the referees on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewers' comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Eric Sawey, PhD
Executive Editor
Life Science Alliance
http://www.lsajournal.org

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A. THESE ITEMS ARE REQUIRED FOR REVISIONS
-- A letter addressing the reviewers' comments point by point.
-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).
-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors
-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.
-- By submitting a revision, you attest that you are aware of our payment policies found here: https://www.life-science-alliance.org/copyright-license-fee

B. MANUSCRIPT ORGANIZATION AND FORMATTING:
Full guidelines are available on our Instructions for Authors page, https://www.life-science-alliance.org/authors
We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

***IMPORTANT: It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.***

Reviewer #1 (Comments to the Authors (Required)):

This manuscript by Lyu et al. describes the implementation of the Build and Retrieve (BaR) methodology to study proteomics of human kidney microsomes. The authors have identified and determined the structure of four different enzymes - the heterodimeric complex glucosidase II (GANAB), retinaldehyde dehydrogenase (ALDH1A1), fructose-bisphosphate adolase (FPA) and betaine-homocysteine methyltransferase (BHMT) - using cryogenic electron microscopy combined with mass spectrometry. These enzymes are directly associated with chronic diseases including diabetes and cardiovascular diseases. Overall, the authors provided comprehensive structural analyses of each protein. Also, this work introduces an integrated approach between proteomics and structural biology which can be useful for studying other endogenous biological samples. I believe that this work is comprehensive and could be a good fit for Life Science Alliance. I have a couple of suggestions/questions for the authors to address:

• The two major peaks on the size exclusion chromatography should be shown in the supplementary figure.

• Each structure reveals some key residues that are involved either in stabilizing the interaction between subunits in the oligomerization, or in substrate binding. However, I would like to see more discussion on what these structures tell us in the context of disease-causing mutations, and how structural information translates into understanding the mechanism of human diseases. Mutations in these particular proteins associating with critical diseases in human health should also be mapped on to the structures.

• It would be great if the authors could share biochemical evidence or references to validate the interaction between the subunits and the substrate/ion binding sites in these enzymes.

• In the structure of GANAB, N97 is likely to be glycosylated. Has any biochemical or functional assay been performed on this residue previously?

• The authors state that the cryo-EM structure is similar to the crystal structure of the mouse homologous enzyme alpha-glucosidase II. The authors should include RMSD, superimposition and sequence similarity between the human and the mouse structures.

• In the GANAB structure, two hexacoordinated Ca2+ ions are found within the beta-subunit. What is the relevance of Ca2+ ions in their enzymatic activity? Has there been any mutagenesis work on the residues that coordinate Ca2+ ions?

• In the processing of the ALDH1A1 dataset, have the authors tried 3D classification or focus refinement in the cofactor-binding site region to attempt to locate an extra density for the NAD+ cofactor?

• In the processing of FPA, have the authors observed any complexes with this enzyme?

• The overall quality of each map is good. However, there seems to be some stretching of the density in the BHMT structure. I recommend including a Euler angle distribution of all particles used in the final map reconstruction in one of the supplementary figures.

Reviewer #2 (Comments to the Authors (Required)):

1. Lyu et al present a concise and beautiful cryo-EM study identifying and visualizing 4 proteins from raw kidney microsomal lysate, GANAB, ALDH1A1, FPA, and BHMT, using the previously-described cryo-EM workflow of Build-and-Retrieve, which includes preparation of cell lysate, protein isolation by SEC, cryo-EM analysis and model building. This work is a testament to the ever-more challenging nature of targets of cryo-EM structural characterization and represents the trajectory of not just visualizing, but also identifying critical cellular complexes and elucidating their functions by cryo-EM.

2. I have no demands or suggestions for additional experiments, merely comments that I believe, if addressed, would strengthen
the manuscript.

Major comments
-This brief and descriptive study relies heavily on work presented by Su et al in Nature Methods. As a result, the manuscript is highly derivative in its nature. Due to the technical aspects of this study, more in-depth methodological details should be included (rather than referenced to in the Su et al).
-It is unclear to me how the SEC run could result in only two peaks that spanned 100-200, and 250-650kDa, respectively; that's the entire separation span of the column. Do they have two macromolecular complexes or is this due to lack of further separation?
-It's unclear to me how particle picking was performed; where all particles in each sample (containing particles ranging in size) picked on were parameters used to only pick certain particles?
-Initial versus final 2D should be shown to illustrate particle heterogeneity and provide further insight into the BnR process.
-Is it unclear exactly how the symmetry was determined in the different maps.

Minor
-EM density maps are referred to as "high-quality", a more adequate description is "high-resolution.
-Were the maps validated using other programs, e.g. Relion?

3. I would like to see data from the sample preparation presented.

Reviewer #3 (Comments to the Authors (Required)):

The present manuscript follows a publication by the same group last year (Su et al, 2021). In that publication, the authors developed the "Build and Retrieve (BaR)" method, which enables the determination of cryo-EM structures of multiple proteins simultaneously from a heterogeneous protein sample. In this manuscript, utilizing BaR method, the authors solved high resolution cryo-EM structures of four kidney enzymes at one time from raw human kidney microsomal lysate. BaR method is indeed a useful tool to help researchers overcome homogeneity and purity problems of sample preparation. My major concern is the "efficiency" of this method since we do not know what protein structure will be solved until the last moment. For example, among the four structures solved by authors, the structures of human FPA and BHMT have been determined by crystallization previously. It is worthwhile to discuss the possible limitations of the BaR method and potential solution, if there is, in the discussion section. In sum, this work proved the feasibility of solving the atomic resolution structures of proteins from raw samples, I recommend publication after the below points are addressed.

1. Show gel filtration chromatography and SDS-PAGE of the purified samples in supplementary figure, label the fractions which applied to cryo-EM. The authors should highlight the bands or areas of the four proteins on SDS-PAGE gel to help readers visualize the proportion of target proteins in the mixture.

2. I would appreciate it if the box in the figure which used to show a close-up view can be considerably obvious.
Reviewer #1 (Comments to the Authors (Required)):

This manuscript by Lyu et al. describes the implementation of the Build and Retrieve (BaR) methodology to study proteomics of human kidney microsomes. The authors have identified and determined the structure of four different enzymes - the heterodimeric complex glucosidase II (GANAB), retinaldehyde dehydrogenase (ALDH1A1), fructose-bisphosphate adolase (FPA) and betaine-homocysteine methyltransferase (BHMT) - using cryogenic electron microscopy combined with mass spectrometry. These enzymes are directly associated with chronic diseases including diabetes and cardiovascular diseases. Overall, the authors provided comprehensive structural analyses of each protein. Also, this work introduces an integrated approach between proteomics and structural biology which can be useful for studying other endogenous biological samples. I believe that this work is comprehensive and could be a good fit for Life Science Alliance. I have a couple of suggestions/questions for the authors to address:

We really appreciate this reviewer’s recommendation, indicating that “this work is comprehensive and could be a good fit for Life Science Alliance”.

• The two major peaks on the size exclusion chromatography should be shown in the supplementary figure.

A new supplementary figure (Fig. S1) showing the two peaks from size exclusion chromatography has been included in this revised manuscript.

• Each structure reveals some key residues that are involved either in stabilizing the interaction between subunits in the oligomerization, or in substrate binding. However, I would like to see more discussion on what these structures tell us in the context of disease-causing mutations, and how structural information translates into understanding the mechanism of human diseases. Mutations in these particular proteins associating with critical diseases in human health should also be mapped on to the structures.

We have included several paragraphs, such as “These residues at the subunit-subunit interface could be very important for the function of GANAB, where a mutation of one or more of these residues could lead to devastating illnesses. For example, a missense mutation R817W has been identified in three patients with autosomal-dominant polycystic liver disease (Porath et al, 2016). Based on our cryo-EM structure, R817 is critical for stabilizing the interaction between the α and β subunits, as this α-subunit residue directly contacts N93 of the β-subunit to form a hydrogen bond (Figure 1D).” (p. 7), “These subunit-subunit interface residues are likely important for the function this enzyme. Interestingly, two missense mutations, A151S and I157T, have been identified for the human ALDH1A2 enzyme (Christy & Doss, 2015). These two mutations are strongly associated with congenital heart disease. The corresponding two amino acids in ALDH1A1 are the interface residues A134 and I140 (Figure 2C), where they may be critical for the tetrameric oligomerization of this enzyme.” (p. 9), “Hereditary fructose intolerance is an autosomal recessive disease caused by the catalytic deficiency of FPA. Several missense mutations have been identified that are directly linked to this disease (Tolan, 1995). One of these missense mutants is the conversion of L257 to a proline (Tolan, 1995). This residue is located at the subunit-subunit interface to strengthen oligomerization (Figure 3C).” (p. 12), and “Alanine
scanning mutagenesis suggests that H338, R346 and W352, located at the subunit-subunit interfaces of the human BHMT enzyme (Figure 4C), are critical residues (Szegedi & Garrow, 2004) Mutations of these individual residues abolished enzymatic activity, suggesting that subunit-subunit interactions may be a prerequisite for the function of this enzyme.” (p. 14) in this revised manuscript to highlight the importance of these residues at subunit-subunit interfaces of these enzymes. Mutations of these interface residues could cause devastating diseases.

- It would be great if the authors could share biochemical evidence or references to validate the interaction between the subunits and the substrate/ion binding sites in these enzymes.

In this revised version of the manuscript, we have included several paragraphs, including

“Interestingly, a study of the homologous α-glucosidase II enzyme from Schizosaccharomyces pombe using alanine scanning mutagenesis indicated that mutations of residues E73 and E114 of the β-subunit inactivated its function. This work underscored the importance of these glutamates to coordinate with Ca2+ ions. The two corresponding residues in the human kidney GANAB enzyme are E64 and E105 which are also responsible for anchoring Ca2+ ions (Figure 1F).” (p. 7), “ALDH1A1 has been found to effectively increase NADH levels and promote tumor growth. Residues involved in creating the NAD+–binding site are presumed to be critical for the function of this enzyme. Indeed, it has been observed that cells harboring a mutation of the conserved interface residue K193 (K193Q or K193R) of ALDH1A1 are much less tumorigenic when compared with cells carrying the wild-type ALDH1A1 enzyme (Liu et al., 2021). Further, another mutagenesis study indicated that the activity of ALDH1A1 was significantly reduced when two glycine residues located at the NAD+–binding site, G246 and G251, were replaced by alanines (Wang et al., 2017), probably due to the effect of steric hindrance.” (p. 10), “Several studies have shown that the glutamate and lysine residues within the substrate-binding site are critical for the function of FPA enzymes (Gupta et al., 1993; Hartman & Brown, 1976; Lai et al., 1974; Lobb et al., 1975; Morris & Tolan, 1993, 1994). Interestingly, a patient with hereditary fructose intolerance was found to have a six-nucleotide deletion in exon 6. This deletion perturbs the position and orientation of the corresponding K147 and E188 residues of human FPA at the active site (Figure 3F) (Santamaria et al., 1999). In addition, a separate study depicted that R304 (Figure 3F) changed to a tryptophan at this catalytic site gives rise to hereditary fructose intolerance (Tolan, 1995). This missense mutation probably diminishes the binding of substrates and leads to this disease.” (p. 12), and “Interestingly, an experimental study using recombinant human liver BHMT suggested that the three cysteine residues, C217, C299 and C300 (Figure 4F), are critical for Zn2+ binding. A mutation of any of these cysteines results in complete loss of activity of this enzyme (Breksa & Garrow, 1999). Further, using rat liver BHMT, it was found that mutations of residues corresponding to substrate-binding residues of the human enzyme, including D26, Y77 and E159 (Figure 4F), significantly depletes enzyme activity (González et al., 2003).” (p. 15) to highlight the importance of residues located at the binding sites of these enzymes.

- In the structure of GANAB, N97 is likely to be glycosylated. Has any biochemical or functional assay been performed on this residue previously?

Yes, it has been found that GANAB is glycosylated. A few statements “This observation is indeed in good agreement with results from a biochemical study that determined that human
GANAB is glycosylated (Martiniuk et al., 1985). Interestingly, the x-ray structure of mouse α-glucosidase II also depicts that this enzyme is glycosylated at residue N97 with the same elongated glycan chain (Caputo et al., 2016).” have been included in this revised version of the manuscript (p. 6) to address this.

• The authors state that the cryo-EM structure is similar to the crystal structure of the mouse homologous enzyme alpha-glucosidase II. The authors should include RMSD, superimposition and sequence similarity between the human and the mouse structures.

We thank this reviewer for this constructive comment. A new paragraph “Our cryo-EM structure indicates that human kidney GANAB is composed of one α-subunit and one β-subunit (Figure 1A), which is in good agreement with the crystal structure of the homologous mouse enzyme α-glucosidase II (Caputo et al., 2016). Protein sequence alignment shows that these two enzymes share 96% protein sequence similarity. Superimposition of the cryo-EM structure of the human enzyme to the x-ray structure of the mouse enzyme (PDB ID: 5F0E) (Caputo et al., 2016) gives rise to a root-mean-square-deviation (r.m.s.d.) of 0.52 Å (for 850 Cα atoms).” has been added on p. 5 of this revised manuscript to address this comment.

• In the GANAB structure, two hexacoordinated Ca2+ ions are found within the beta-subunit. What is the relevance of Ca2+ ions in their enzymatic activity? Has there been any mutagenesis work on the residues that coordinate Ca2+ ions?

Yes, a mutagenesis study on a homologous enzyme from Schizosaccharomyces pombe has been done, indicating that the glutamate residues responsible for anchoring bound Ca2+ ions are important for the function. A statement “Interestingly, a study of the homologous α-glucosidase II enzyme from Schizosaccharomyces pombe using alanine scanning mutagenesis indicated that mutations of residues E73 and E114 of the β-subunit inactivated its function. This work underscored the importance of these glutamates to coordinate with Ca2+ ions. The two corresponding residues in the human kidney GANAB enzyme are E64 and E105 which are also responsible for anchoring Ca2+ ions (Figure 1F).” has been added on p.7 of the revised manuscript to address this.

• In the processing of the ALDH1A1 dataset, have the authors tried 3D classification or focus refinement in the cofactor-binding site region to attempt to locate an extra density for the NAD+ cofactor?

Yes, we tried both the 3D classification and focused refinement at the cofactor-binding site region. We did not observe any extra densities corresponding to the bound NAD+ cofactor.

• In the processing of FPA, have the authors observed any complexes with this enzyme?

We also tried both the 3D classification and focused refinement at the substrate-binding site. Again, we did not observe any extra densities corresponding to bound substrate.

• The overall quality of each map is good. However, there seems to be some stretching of the density in the BHMT structure. I recommend including a Euler angle distribution of all particles
used in the final map reconstruction in one of the supplementary figures.

We have included Euler angle distributions for all structures in the supplementary figures (Figs. S3-S6)

Reviewer #2 (Comments to the Authors (Required)):

1. Lyu et al present a concise and beautiful cryo-EM study identifying and visualizing 4 proteins from raw kidney microsomal lysate, GANAB, ALDH1A1, FPA, and BHSMT, using the previously-described cryo-EM workflow of Build-and-Retrieve, which includes preparation of cell lysate, protein isolation by SEC, cryo-EM analysis and model building. This work is a testament to the ever-more challenging nature of targets of cryo-EM structural characterization and represents the trajectory of not just visualizing, but also identifying critical cellular complexes and elucidating their functions by cryo-EM.

We thank this reviewer’s glowing comment, indicating that this is “a concise and beautiful cryo-EM study” and “a testament to the ever-more challenging nature of targets of cryo-EM structural characterization and represents the trajectory of not just visualizing, but also identifying critical cellular complexes and elucidating their functions by cryo-EM”.

2. I have no demands or suggestions for additional experiments, merely comments that I believe, if addressed, would strengthen the manuscript.

Major comments
-This brief and descriptive study relies heavily on work presented by Su et al in Nature Methods. As a result, the manuscript is highly derivative in its nature. Due to the technical aspects of this study, more in-depth methodological details should be included (rather than referenced to in the Su et al).

The Build-and-Retrieve procedures, starting from initial 2D classification to final map construction, have been rewritten in the “Method” section of this revised manuscript to describe the details of the protocol, following the reviewer’s comment.

-It is unclear to me how the SEC run could result in only two peaks that spanned 100-200, and 250-650kDa, respectively; that's the entire separation span of the column. Do they have two macromolecular complexes or is this due to lack of further separation?

Our goal for using SEC is to enrich protein particles, allowing us to obtain more particle counts. We only focus on particles with size $\geq 100$ kDa, which would make it easier for high-resolution cryo-EM structural determination. During the enrichment process, we were also able to separate the large peak corresponding to aggregation from protein particles. For size $\geq 100$ kDa, we only observed two peaks, 100-200 kDa and 250-650 kDa, from our sample (see Fig. S1).

-It's unclear to me how particle picking was performed; where all particles in each sample
(containing particles ranging in size) picked on were parameters used to only pick certain particles?

The initial particle stack was picked by using Topaz (Bepler et al, 2019, 2020) with the default ResNet16 (64 units) pretrained model (which has been trained on a large corpus of datasets including a wide variety of particles).

-Initial versus final 2D should be shown to illustrate particle heterogeneity and provide further insight into the BnR process.

The initial 2D images have been included in Fig. S2 (Build and Retrieve workflow) of the supplementary materials of this revised manuscript. The selected particles of GANAB, ALDH1A1, FPA, and BHMT are highlighted with magenta hexagons, yellow squares, pink circles, and green stars, respectively, in this supplementary figure. The final 2D images of these enzymes are included in Figs. S3B, S4B, S5B and S6B.

-Is it unclear exactly how the symmetry was determined in the different maps.

We first applied C1 symmetry to each protein. We then determined the symmetry using Chimera. The criterion is that the correlation of the maps before and after a particular symmetry transformation has to be ≥ 0.99 to ensure that the symmetry is a correct.

Minor
-EM density maps are referred to as "high-quality", a more adequate description is "high-resolution.

The use of “high-quality” to describe cryo-EM maps has been switched to “high-resolution”, following this reviewer’s suggestion.

-Were the maps validated using other programs, e.g. Relion?

Yes, the resolutions of the maps were validated using RELION. For example, the resolutions of the maps corresponding to GANAB, ALDH1A1, FPA and BHMT were reported 2.88 Å, 2.84 Å, 2.80 Å and 2.62 Å, respectively, in cryoSPARC. These resolutions became 2.89 Å, 2.86 Å, 2.84 Å and 2.65 Å, respectively, from RELION. A statement “The resolutions of these maps were also verified using RELION (Scheres, 2012)” has been added in the method section of this revised manuscript.

3. I would like to see data from the sample preparation presented.

In this revised manuscript, we have included a new supplementary figure (Fig. S1), showing the SEC peaks for sample enrichment and SDS-PAGE of these two SEC peaks.

Reviewer #3 (Comments to the Authors (Required)):
The present manuscript follows a publication by the same group last year (Su et al, 2021). In that publication, the authors developed the "Build and Retrieve (BaR)" method, which enables the determination of cryo-EM structures of multiple proteins simultaneously from a heterogeneous protein sample. In this manuscript, utilizing BaR method, the authors solved high resolution cryo-EM structures of four kidney enzymes at one time from raw human kidney microsomal lysate. BaR method is indeed a useful tool to help researchers overcome homogeneity and purity problems of sample preparation. My major concern is the "efficiency" of this method since we do not know what protein structure will be solved until the last moment. For example, among the four structures solved by authors, the structures of human FPA and BHMT have been determined by crystallization previously. It is worthwhile to discuss the possible limitations of the BaR method and potential solution, if there is, in the discussion section. In sum, this work proved the feasibility of solving the atomic resolution structures of proteins from raw samples, I recommend publication after the below points are addressed.

We very much appreciate this reviewer’s recommendation for the possibility of publishing our manuscript in Life Science Alliance. A discussion regarding possible limitations of the BaR methodology has been included in the last paragraph of the “Discussion” section of this revised version of the manuscript.

1. Show gel filtration chromatography and SDS-PAGE of the purified samples in supplementary figure, label the fractions which applied to cryo-EM. The authors should highlight the bands or areas of the four proteins on SDS-PAGE gel to help readers visualize the proportion of target proteins in the mixture.

A new supplementary figure (Fig. S1), showing the two SEC peaks and their corresponding SDS-PAGE, has been added in this revised version of the manuscript, following this reviewer’s suggestion.

2. I would appreciate it if the box in the figure which used to show a close-up view can be considerably obvious.

We thank for this reviewer’s suggestion. The boxes in the figures have been remade, making them easier to see.
October 26, 2022

RE: Life Science Alliance Manuscript #LSA-2022-01580-TR

Prof. Edward W. Yu
Case Western Reserve University
Department of Pharmacology
Cleveland, Ohio 44106

Dear Dr. Yu,

Thank you for submitting your revised manuscript entitled “Simultaneous solving high-resolution structures of various enzymes from human kidney microsomes”. We would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines.

Along with points mentioned below, please tend to the following:
- please address Reviewer 1’s final comments
- please upload your supplementary figure files as single files and add your supplementary figure files to the main manuscript text
- please add the Twitter handle of your host institute/organization as well as your own or/and one of the authors in our system
- please add the author contributions and a conflict of interest statement to the main manuscript text
- please use the [10 author names, et al.] format in your references (i.e. limit the author names to the first 10)
- please upload your table files as separate editable excel or doc files or make sure that they’re included in the doc file of your manuscript
- the pdb accession numbers and EMDB accession codes should now be made publicly accessible

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

LSA now encourages authors to provide a 30-60 second video where the study is briefly explained. We will use these videos on social media to promote the published paper and the presenting author (for examples, see https://twitter.com/LSAjournal/timelines/143745065917124608). Corresponding or first-authors are welcome to submit the video. Please submit only one video per manuscript. The video can be emailed to contact@life-science-alliance.org

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You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. FINAL FILES:

These items are required for acceptance.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://www.life-science-alliance.org/authors

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary “Source Data” files.
**Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.**

**It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.**

**The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements.**

**Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.**

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Eric Sawey, PhD  
Executive Editor  
Life Science Alliance  
http://www.lsajournal.org

Reviewer #1 (Comments to the Authors (Required)):

The revised manuscript by Lyu et al., has been improved by addressing the major concerns raised in previous review of the original manuscript. Also, the figures are improved and easy to follow. The authors have provided comprehensive cryo-EM structural analyses of four important enzymes isolated from human kidney microsomes in context of human diseases. The Build and Retrieve approach has proven to be an effective tool for studying challenging targets from raw biological samples with a significant potential to advance the field of structural biology. I remain supportive for the work to be published in Life Science Alliance after the minor issues to be addressed.

In page 16, the paragraph contains a repetition 'of protein biosynthesis and quality control...'.

The word 'Interestingly' appears too frequently, and can be omitted or substituted with other words.
-please add a conflict of interest statement to the main manuscript text

The conflict of interest statement has been included in this revised manuscript.

-the pdb accession numbers and EMDB accession codes should now be made publicly accessible

We have requested to release all PDBs and EMDBs and made them available to the public.
Dear Dr. Yu,

Thank you for submitting your Research Article entitled “Simultaneous solving high-resolution structures of various enzymes from human kidney microsomes”. It is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

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Scheduling details will be available from our production department. You will receive proofs shortly before the publication date. Only essential corrections can be made at the proof stage so if there are any minor final changes you wish to make to the manuscript, please let the journal office know now.

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Authors are required to distribute freely any materials used in experiments published in Life Science Alliance. Authors are encouraged to deposit materials used in their studies to the appropriate repositories for distribution to researchers.

You can contact the journal office with any questions, contact@life-science-alliance.org

Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Eric Sawey, PhD
Executive Editor
Life Science Alliance
http://www.lsajournal.org