

Peer Review File

Manuscript Title: A self-healing multispectral transparent adhesive peptide glass

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1:

Remarks to the Author:

This work presents the discovery of a novel and interesting glassy material based on a hydrated tyrosine tripeptide. The glassy nature is demonstrated by x-ray diffraction patterns, by calorimetry, and optical appearance such as large spectral window transparency. A number of interesting applications and properties are mentioned, e.g., the observed self healing effect.

In my view, this paper should be published in Nature Materials, after the authors have acted on the following points:

- 1 - The first half of the abstract is a general description of glassy materials, which can be shortened. And the abstract should mention the specific material this paper is about.
- 2 - Regarding the simple lens production, I have not seen a reason why they would not dry up and crack when subject to ambient conditions.
- 3 - The work mentions the strong adhesion to a glass slide, but does not mention how that slide surface had been treated prior to the adhesion experiment.
- 4 - the statement 'exposed to extremely dry conditions' needs to be quantified by values for humidity and pressure. Similarly, 'dry environment' and 'humid' environment' have to be quantified.

Referee #2:

Remarks to the Author:

There is an active search worldwide towards new glassy materials, but this is a big challenge that hasn't yet found viable alternatives to revolutionize our society with, let's say, eco-friendly and innovative, viable alternatives to glass. This work by Gazit and co-workers tackles this challenge and provides a very interesting potential solution.

They reported the study of the self-assembly of a tripeptide YYY into an amorphous rigid glass. The hydration of the peptide by a humid environment induces the formation of strong non-covalent cross-linking by water molecules and the formation of supramolecular glass. The glass shows excellent mechanical properties, including a remarkable self-healing ability and a broad range of transparency that makes it an intriguing material for optical applications. Overall, this MS has the potential to influence thinking of the field and to bring on an important advancement of the understanding of how to design new alternatives to glass, to open up new horizons in the area of biomolecular and biodegradable commodity materials that can revolutionize the world. However, to do so, the following

concerns must be addressed:

1. How did the authors come about to the choice for YYY? How many Tyr are needed? The work would be far more impactful if the authors could study let's say Y, YY, YYYY, YYYYY. The choice for Tyr is well explained, but the molecular size is not.

2. Furthermore, often C-terminal amidation results in better properties of peptide materials that rely on H-bonding. The authors should prepare YYY-NH₂ and compare it against YYY.

3. The authors do not discuss the effect of pH, which is a key parameter for a material that depends on the presence of water. Have they tried to prepare the material with water at different pH values? Also in this case, C-terminal amidation may help in extending the range of stability of the material by reducing the supramolecular effects due to the ionization state of the peptide at different pH values.

4. It is unclear how the peptide was sourced (commercially available?). In any case, mass spec itself is not a sufficient proof of purity, which is a crucial parameter for supramolecular studies and for their reproducibility, as well as its ionization state and presence of counterions. The authors should clearly indicate if the peptide was bought: the commercial provider, the declared purity level, at the very least LC-MS trace and purification method by the commercial supplier (i.e., precipitation from organic solvent? HPLC in formic acid or TFA?), and ideally elemental analysis. OR, if the peptide was made, ¹H and ¹³C NMR, mono and bidimensional and correlative to clearly assign all the signals.

5. The authors chose a peptide concentration of 100 mg/mL, why? Is it the solubility limit of the tripeptide? It could be interesting to understand if there is any influence of the concentration on the mechanical and optical properties of the glass. The correspondence in molarity is strongly recommended as an addition, and of course this will heavily depend on the presence of counterions or impurities that will become evident addressing point 4 above. This is crucial for reproducibility of the study.

6. Importantly, the authors described and studied the effect of water molecules on the mechanical properties. However, it is not clear from the paper how it could be possible to reproduce the experiments. For example, they observed a different behaviour under a humid or dry environment. Unfortunately, they did not specify the level of humidity, which is thus likely to have a strong influence on the self-assembly process and on the properties of the material. Did the authors perform experiments under controlled conditions of temperature and humidity? I think that authors should add in the Methods section the detailed procedure to prepare the material and additional experiments at controlled temperature and humidity, otherwise it will simply be not possible for others to reproduce this exciting science.

7. Raman spectroscopy, conducted over the entire phase transition process, could be a useful technique to further probe the glass transition mechanism. Please check the ref. 10.1126/sciadv.add8105.

8. There are some typos in the manuscript. 96 “npeptide – nglass ≈ 0.01 ” wrong font. Caption of Fig 2-175 (DMSO-d8), correct in DMSO-d6. Fig 3 “Glass transssition”, typo glass transition. 224, “ 747 ± 120 ”, and 225, “ $Er = 12 \pm 1$ ”, wrong font, etc.

Referee #3:

Remarks to the Author:

The submitted work shows glass-forming properties by a tyrosine tripeptide, in which the amount of water present can also modulate physical properties by acting as a plasticizer.

The unique properties of the glass and its potential application make the work appealing for publication in Nature. The experiments are appropriate for the physical characterization of the material.

That said, before the work can be published, I feel like the following points should be addressed, or at least considered:

1- It would be nice to show the tripeptide's structure in a scheme somewhere in the introduction. Also, I assume that it's the zwitterion and that there are no additional counter-ions, but it would also be nice to specify it.

2- p3, l49-51: this is correct for glasses with high Tg, but glasses with near-ambient Tg can self-heal spontaneously. Likewise, glasses can be healed by heating them close to Tg, provided that they do not crystallize in the process. The sentence should be modified to reflect that.

3- It is revealed on p. 10 that the properties of glass depend on the amount of water present: the two DSC scans in Figs. 1 and 3 showing Tg values of 37 oC before heating to dehydrate, and 119 oC after dehydration. Ideally, the two DSC scans should be put in the same figure to illustrate the change due to the amount of water present, otherwise the results seem somewhat disjointed. It has been correctly asserted that water acts as a plasticizer that lowers Tg.

4- Perhaps the most important point to consider, the authors specified that glass formation by peptides was extremely rare. However, have the authors studied other short tyrosine peptides? For example, YY, YYYY and YYYYY, to see if those exhibited any glass-forming properties? I feel like it would be crucial to run quick tests to ensure that it is unique to YYY and not common among short Y oligomers. If this property is indeed unique to YYY, it raises another very important question: why only the trimer and not other oligomers, which would probably constitute the basis for future work.

Author Rebuttals to Initial Comments:

Referees' comments:

Referee #1 (Remarks to the Author):

Referee #1: This work presents the discovery of a novel and interesting glassy material based on a hydrated tyrosine tripeptide. The glassy nature is demonstrated by x-ray diffraction patterns, by calorimetry, and optical appearance such as large spectral window transparency. A number of interesting applications and properties are mentioned, e.g., the observed self healing effect. In my view, this paper should be published in Nature Materials, after the authors have acted on the following points:

Response: We thank the referee for the positive and encouraging assessment of our work and for proposing useful comments, which we feel have contributed to improving our manuscript.

Referee #1: 1 - The first half of the abstract is a general description of glassy materials, which can be shortened. And the abstract should mention the specific material this paper is about.

Response: We thank the reviewer for bringing this issue to our attention. The abstract was revised as suggested and now reads as follows (page 2, line 33):

“Despite its disordered liquid-like structure, glass exhibits solid-like mechanical properties¹. The formation of glassy material occurs by vitrification, preventing crystallization and promoting an amorphous structure². Glass is fundamental in diverse fields of materials science, owing to its unique optical, chemical, and mechanical properties as well as durability, versatility, and environmental sustainability³. However, engineering a glassy material without compromising its properties is a major challenge⁴⁻⁶. Here, we report the discovery of a supramolecular amorphous glass formed by the spontaneous self-organization of the short aromatic tri-peptide YYY initiated via noncovalent cross-linking with structural water^{7,8}. This system uniquely combines often contradictory sets of properties; it is highly rigid yet can undergo complete self-healing at room temperature. In addition, the supramolecular glass is an extremely strong adhesive yet it is transparent in a wide spectral range from visible to mid-infrared. This exceptional set of characteristics is observed in a simple bioorganic peptide glass composed of natural amino acids, presenting a multi-functional material that could be highly advantageous for various applications in science and engineering.”

Referee #1: 2 - Regarding the simple lens production, I have not seen a reason why they would not dry up and crack when subject to ambient conditions.

Response: We thank the referee for allowing us to better explain this point. While it is true that the lens can be subjected to damage and cracking due to dry environment conditions, it can also be 'self-healed' with exposure to humid conditions. In order to maintain the proper function of these lenses, the surrounding environment of the lens should be regulated or constrained. Encasing these lenses in a confined, closed system should enable prolonged performance of the lens. In order to emphasize this point, the text has been revised and now reads as follows (page 19, line 437):

"In order to prolong the shelf-life of such lenses, it can be beneficial to encase the lenses to prevent damage from changing humidity conditions, although such damages were not visible at ambient conditions through the span of several weeks."

Referee #1: 3 - The work mentions the strong adhesion to a glass slide, but does not mention how that slide surface had been treated prior to the adhesion experiment.

Response: We thank the referee for pointing out this important issue. Prior to the application of the peptide solution, the glass slide surface, a simple microscope glass slide, was cleaned with ethanol, and water and wiped dry with a technical cleanroom cloth. In order to emphasize that there is no need for any surface treatment or activation of the surface, the manuscript was revised and now reads as follows (page 18, line 394):

"Microscope slides (HDA, CAS 7102) were cleaned and dried using ethanol, water and TechniCloth (Texwipe TX 604) prior to the application of the peptide solution."

Referee #1: 4 - the statement 'exposed to extremely dry conditions' needs to be quantified by values for humidity and pressure. Similarly, 'dry environment' and 'humid' environment' have to be quantified.

Response: We thank the referee for allowing us to clarify this point. To address this, we have added a paragraph to the methods section describing the relevant experiments with quantitative values. The manuscript was revised and now reads as follows (page 21, line 468):

"Cracking and self-healing of the peptide glass:

Humid conditions were obtained by using an Okolab H301 incubating chamber. The measurements were conducted at 23°C, while the offset was set to zero, resulting in a relative humidity of 85%. Dry conditions were obtained by adding 30 g of desiccant silica gel orange

(Thermo, #7631-86-9) to a sealed and disabled incubating chamber, reaching a relative humidity of 20%, as measured by a hygrometer (HTC-1). The video frames were captured using a Zyla 4.2 sCMOS camera (Andor Technology, Northern Ireland) mounted on an Eclipse Ti-E inverted optical microscope (Nikon, Japan).”

Referee #2 (Remarks to the Author):

Referee #2: There is an active search worldwide towards new glassy materials, but this is a big challenge that hasn't yet found viable alternatives to revolutionize our society with, let's say, eco-friendly and innovative, viable alternatives to glass. This work by Gazit and co-workers tackles this challenge and provides a very interesting potential solution.

They reported the study of the self-assembly of a tripeptide YYY into an amorphous rigid glass. The hydration of the peptide by a humid environment induces the formation of strong non-covalent cross-linking by water molecules and the formation of supramolecular glass. The glass shows excellent mechanical properties, including a remarkable self-healing ability and a broad range of transparency that makes it an intriguing material for optical applications. Overall, this MS has the potential to influence thinking of the field and to bring on an important advancement of the understanding of how to design new alternatives to glass, to open up new horizons in the area of biomolecular and biodegradable commodity materials that can revolutionize the world. However, to do so, the following concerns must be addressed:

Response: We thank the referee for providing a positive summary of the main points of this study and their innovative nature in addition to providing the useful suggestions below, which greatly contributed to an improved manuscript.

Referee #2: 1 - How did the authors come about to the choice for YYY? How many Tyr are needed? The work would be far more impactful if the authors could study let's say Y, YY, YYYY, YYYYY. The choice for Tyr is well explained, but the molecular size is not.

Response: We appreciate the opportunity given by referee #2 to expand on the rational study that led us to the discovery of the peptide glass. The initial study was prompted by the question of solubility of Phenylalanine (F) and Tyrosine (Y) amino acids and related short peptides. While the amino acid F has a solubility of ~30 mg/ml in water, the supposedly more hydrophilic Y amino acid, which has the same structure as F only with an additional hydrophilic hydroxyl group, has a much lower solubility of ~0.45 mg/ml in water. The decreased solubility of the Y amino acid is explained in a prior work published by our group (10.1021/acsnano.9b08217). However, when looking at di-peptides, FF (~0.76 mg/ml) is much less soluble than YY (~1000 mg/ml). This led us

to investigate the solubility of all F and Y related peptides, up to three amino acids. To our surprise, out of all the amino acids and peptides tested, only YYY exhibited an amorphous structure following assembly from an aqueous solution. This then led us to test YYYY as well, and we revealed that it has a crystalline powder x-ray diffraction. For this reason, we focused only on YYY. However, we agree with the referee that the questions regarding the rationality of the study come to mind when reading about the extraordinary properties of YYY. Hence, we included some of the results in the manuscript to answer these questions and the manuscript now reads as follows (page 4, line 84):

“While systematically studying short peptides with repeating tyrosine units, we discovered that a tyrosine tripeptide (YYY) is anomalously highly soluble and spontaneously assembles into an amorphous rigid glass by evaporation of an aqueous solution, in spite of the aromatic nature of the tyrosine single amino acid, its poor solubility and high propensity to crystallize^{32–34}. Whereas Y/ YY/ YYYY formed crystals with a defined crystallographic pattern (Supplementary Fig. 1)”

Referee #2: 2 - Furthermore, often C-terminal amidation results in better properties of peptide materials that rely on H-bonding. The authors should prepare YYY-NH₂ and compare it against YYY.

Response: We Thank the referee for giving us the opportunity to deepen our understanding of the mechanism of the supramolecular structure formation. To answer this question, YYY-NH₂ was synthesized. When a solution of 100 mg/ml of YYY-NH₂ was allowed to evaporate, the assembly adhered to the microscope glass slide. In addition, PXRD results indicated that it has an amorphous structure as glass, showing the same behavior as YYY. The manuscript was revised to include these results and now reads as follows (page 8, line 173):

“To further study the role of intermolecular H-bonds in the formation of the supramolecular structure, an amidated derivative of the peptide (YYY-NH₂) was synthesized. When a solution of 100 mg/ml YYY-NH₂ was allowed to evaporate, the assembly adhered to the microscope glass slide and had an amorphous structure characteristic of glass as confirmed by PXRD (Supplementary Fig. 9), thus showing the same behavior as YYY.”

Referee #2: 3 - The authors do not discuss the effect of pH, which is a key parameter for a material that depends on the presence of water. Have they tried to prepare the material with water at different pH values? Also in this case, C-terminal amidation may help in extending the range of stability of the material by reducing the supramolecular effects due to the ionization state of the peptide at different pH values.

Response: We thank the referee for providing us with the opportunity to improve our study and we agree with the referee that the effect of pH on the assembly was lacking in our manuscript. Prompted by the referee's comment, we tested the assembly of YYY under alkaline and acidic conditions (pH = 2, 7, and 10) and discovered that the peptide glass is formed in all of these pH conditions. This observation revealed broad stability of the glassy material assembly. The manuscript was revised to include these results and now reads as follows (page 8, line 177):

“Moreover, to examine the effect of the peptide ionization state on its self-assembly, the peptide was allowed to assemble under alkaline and acidic conditions. Samples prepared in solvents of different pH levels (pH = 2, 7, and 10) were confirmed to be amorphous by PXRD (Supplementary Fig. 10), indicating broad stability of the glassy matter assembly.”

Referee #2: 4 - It is unclear how the peptide was sourced (commercially available?). In any case, mass spec itself is not a sufficient proof of purity, which is a crucial parameter for supramolecular studies and for their reproducibility, as well as its ionization state and presence of counterions. The authors should clearly indicate if the peptide was bought: the commercial provider, the declared purity level, at the very least LC-MS trace and purification method by the commercial supplier (i.e., precipitation from organic solvent? HPLC in formic acid or TFA?), and ideally elemental analysis. OR, if the peptide was made, ¹H and ¹³C NMR, mono and bidimensional and correlative to clearly assign all the signals.

Response: We thank the referee for pointing out this important issue. To answer this, we have added a supplementary figure with the information from the commercial analysis of the peptide synthesis. We also included a ‘Material’ section that reads as follows (page 16, line 322):

“YYY was commercially purchased, purified by high-performance liquid chromatography to >95%, characterized by mass spectroscopy, and supplied as a lyophilized powder by GenScript (Piscataway, NJ). Additional information can be found in Figure S16.”

Referee #2: 5 -The authors chose a peptide concentration of 100 mg/mL, why? Is it the solubility limit of the tripeptide? It could be interesting to understand if there is any influence of the concentration on the mechanical and optical properties of the glass. The correspondence in molarity is strongly recommended as an addition, and of course this will heavily depend on the presence of counterions or impurities that will become evident addressing point 4 above. This is crucial for reproducibility of the study.

Response: We thank the referee for allowing us to further elaborate on this point. YYY was found to be highly soluble, it can be dissolved even up to 1000 mg/ml. The glassy nature of the assembly was confirmed to be amorphous by PXRD regardless of the initial peptide concentration. Moreover, the initial concentration did not affect the mechanical properties of the glass. DSC measurements of the glass assembled by evaporation of different concentrations in a controlled environment of 23°C, 50% RH revealed similar glass transition temperature. The concentration of 100 mg/ml was evaluated to be optimal in terms of evaporation rate in ambient conditions. We agree with the referee that the information regarding the effect of concentration is missing in our manuscript. Hence, we included these results in the manuscript and the manuscript now reads as follows (page 4, line 96):

“regardless of the initial concentration used to prepare the glass (Supplementary Fig. 3)”

Referee #2: 6 - Importantly, the authors described and studied the effect of water molecules on the mechanical properties. However, it is not clear from the paper how it could be possible to reproduce the experiments. For example, they observed a different behaviour under a humid or dry environment. Unfortunately, they did not specify the level of humidity, which is thus likely to have a strong influence on the self-assembly process and on the properties of the material. Did the authors perform experiments under controlled conditions of temperature and humidity? I think that authors should add in the Methods section the detailed procedure to prepare the material and additional experiments at controlled temperature and humidity, otherwise it will simply be not possible for others to reproduce this exciting science.

Response: We thank the referee for allowing us to clarify this point. To address this, we have added a paragraph to the methods section describing the relevant experiments with quantitative values. The manuscript was revised and now reads as follows (page 21, line 468):

“Cracking and self-healing of the peptide glass:

Humid conditions were obtained by using an Okolab H301 incubating chamber. The measurements were conducted at 23°C, while the offset was set to zero, resulting in a relative humidity of 85%. Dry conditions were obtained by adding 30 g of desiccant silica gel orange (Thermo, #7631-86-9) to a sealed and disabled incubating chamber, reaching a relative humidity of 20%, as measured by a hygrometer (HTC-1). The video frames were captured using a Zyla 4.2 sCMOS camera (Andor Technology, Northern Ireland) mounted on an Eclipse Ti-E inverted optical microscope (Nikon, Japan).”

Referee #2: 7 - Raman spectroscopy, conducted over the entire phase transition process, could

be a useful technique to further probe the glass transition mechanism. Please check the ref. 10.1126/sciadv.add8105.

Response: We thank the referee for providing us with the opportunity to further establish our study. Motivated by the referee's comment, we have used in situ Raman spectroscopy to study the glass transition. The Raman spectra results confirmed the amorphous organization of the peptide glass by the absence of sharp peaks in the low frequency region of the Raman spectra (below 200 cm^{-1}). In addition, Raman spectra obtained at different temperatures confirmed that the amorphous organization of the peptide glass was preserved over the temperature range related to the glass transition. Importantly, the Raman spectra analysis allowed us to validate our suggested supramolecular model. The analysis of the doublet intensity ratio between 850 cm^{-1} to 830 cm^{-1} associated with aromatic fingerprints observed in the Raman spectra of peptides supports that the phenol ring is involved in hydrophilic interactions. According to these results, the manuscript was revised and now reads as follows (page 4, line 93; page 4, line 97; page 7, line 165):

“as well as by the absence of sharp peaks in the low frequency region of its Raman spectrum (Supplementary Fig. 2)³⁵”

“In addition, variable-temperature in situ Raman spectroscopy confirmed that the amorphous nature was preserved over the temperature range related to the glass transition (Supplementary Fig. 4)³⁶.”

“Another supporting evidence of the interactions between the peptide and water molecules in the solid state was obtained based on the Raman spectrum of the peptide glass. The analysis of the doublet intensity ratio between 850 cm^{-1} to 830 cm^{-1} associated with aromatic fingerprints observed in the Raman spectra of peptides revealed a ratio of $\rho=1.8$ (Supplementary Fig. 8). While low values of $0.25 < \rho < 1$ are attributed to $\pi-\pi$ interactions of stacked phenol rings, ρ values higher than unity are known to be observed when the phenol ring is exposed to aqueous environment, indicating that the phenol is involved in hydrophilic interactions⁴².”

Referee #2: 8 - There are some typos in the manuscript. 96 “ $n_{\text{peptide}} - n_{\text{glass}} \approx 0.01$ ” wrong font. Caption of Fig 2-175 (DMSO-d8), correct in DMSO-d6. Fig 3 “Glass transssition”, typo glass transition. 224, “ 747 ± 120 ”, and 225, “ $E_r = 12 \pm 1$ ”, wrong font, etc.

Response: We thank the referee for bringing this to our attention. These typos were fixed, as suggested.

Referee #3 (Remarks to the Author):

Referee #3: The submitted work shows glass-forming properties by a tyrosine tripeptide, in which the amount of water present can also modulate physical properties by acting as a plasticizer. The unique properties of the glass and its potential application make the work appealing for publication in Nature. The experiments are appropriate for the physical characterization of the material. That said, before the work can be published, I feel like the following points should be addressed, or at least considered:

Response: We would like to thank the referee for the positive assessment of our work and for the useful comments that helped make the manuscript clearer.

Referee #3: 1 - It would be nice to show the tripeptide's structure in a scheme somewhere in the introduction. Also, I assume that it's the zwitterion and that there are no additional counter-ions, but it would also be nice to specify it.

Response: We agree with the referee that a schematic representation of the molecule early on would assist the reader in understanding the manuscript. Hence, we included a molecular scheme in an inset in Figure 1b.

Referee #3: 2 - p3, l49-51: this is correct for glasses with high T_g , but glasses with near-ambient T_g can self-heal spontaneously. Likewise, glasses can be healed by heating them close to T_g , provided that they do not crystallize in the process. The sentence should be modified to reflect that.

Response: We thank the referee for allowing us to better explain this issue. The sentence was revised and now reads as follows (page 3, line 54):

“Due to their usually fixed molecular organization, high molecular weight glassy polymers show remarkable stiffness, but their sluggish molecular diffusion rate requires heating for self-healing⁵. On the other hand, rubbery polymers composed of low molecular weight molecules can self-heal at room temperature due to their high mobility but are soft and deformable. Accordingly, a glassy material that simultaneously shows robust mechanical properties and the ability to self-heal at ambient conditions has been rarely described^{4,6}.”

Referee #3: 3 - It is revealed on p. 10 that the properties of glass depend on the amount of water present: the two DSC scans in Figs. 1 and 3 showing T_g values of 37 °C before heating to dehydrate, and 119 °C after dehydration. Ideally, the two DSC scans should be put in the same figure to illustrate the change due to the amount of water present, otherwise the results seem somewhat disjointed. It has been correctly asserted that water acts as a plasticizer that lowers T_g.

Response: We thank the referee for allowing us to clarify this point. The change in the T_g through DSC is demonstrated in Figure 3f, where the curves represent heating cycles that result in the change on the T_g due to water evaporation during each heating cycle. Figure 3h represents the limit of water evaporation, as there is minimal difference in T_g between the 4th and 5th heating cycles which were performed after prolonged incubation at 120°C. In order to make the DSC plots clearer to the reader, the title of “minimal water content” was added to the plot in Figure 3h and the labels of “4th heating cycle” and “5th heating cycle” were added as well.

Referee #3: 4 - Perhaps the most important point to consider, the authors specified that glass formation by peptides was extremely rare. However, have the authors studied other short tyrosine peptides? For example, YY, YYYY and YYYYY, to see if those exhibited any glass-forming properties? I feel like it would be crucial to run quick tests to ensure that it is unique to YYY and not common among short Y oligomers. If this property is indeed unique to YYY, it raises another very important question: why only the trimer and not other oligomers, which would probably constitute the basis for future work.

Response: We appreciate the opportunity given by referee #3 to expand on this important topic. The initial study was prompted by the question of solubility of Phenylalanine (F) and Tyrosine (Y) amino acids and related short peptides. While the amino acid F has a solubility of ~30 mg/ml in water, the supposedly more hydrophilic Y amino acid, which has the same structure as F only with an additional hydroxyl group, has a much lower solubility of ~0.45 mg/ml in water. The decreased solubility of the Y amino acid is explained in a prior work published by our group ([10.1021/acsnano.9b08217](https://doi.org/10.1021/acsnano.9b08217)). However, when looking at di-peptides, FF (~0.76 mg/ml) is much less soluble than YY (~1000 mg/ml). This led us to investigate the solubility of all F and Y related peptides, up to three amino acids. To our surprise, out of all the amino acids and peptides tested, only YYY exhibited an amorphous structure following assembly from an aqueous solution. This then led us to test YYYY as well, and we revealed that it has a crystalline powder x-ray diffraction. For this reason, we focused only on YYY. However, we agree with the referee that the questions regarding the rationality of the study come to mind when reading about the extraordinary properties of YYY. Hence, we included some of the results in the manuscript to answer these questions and the manuscript now reads as follows (page 4, line 84):

“While systematically studying short peptides with repeating tyrosine units, we discovered that a tyrosine tripeptide (YYY) is anomalously highly soluble and spontaneously assembles into an amorphous rigid glass by evaporation of an aqueous solution, in spite of the aromatic nature of the tyrosine single amino acid, its poor solubility and high propensity to crystallize^{32–34}. Whereas Y/ YY/ YYYY formed crystals with a defined crystallographic pattern (Supplementary Fig. 1)”

Reviewer Reports on the First Revision:

Referees' comments:

Referee #3 (Remarks to the Author):

I am satisfied with the changes made by the authors, and so believe that the manuscript can be published.

However, just some food for thought for the authors for a potential further study. It so turns out that YY and YYYY do not form glasses, whereas YYY does. One has to wonder why mechanistically, and it is highly likely that even-numbered Tyr oligomers undergo some sort of intra- or intermolecular non-covalent interactions between pairs of side chains (either pi-pi stacking or H bonding of the OH groups). If this is the case, then YYY would be left with a single side chain not interacting, which might frustrate its crystallization. It would be nice to verify this with YYYYY in a future study because it would also have one unpaired side chain. Of course, its higher size might prevent it, but it's worth the try at least.