**Reply to the Review Report (Reviewer 2):**
We would like to thank Reviewer 1 for her/his insightful comments and suggestions. The following is our point-by-point responses to the concerns raised by the reviewer 2.

**Reviewer 2**

**Comments and Suggestions for Authors**

**Comment:**
In the present paper, the authors report on the fabrication of sample substrates for measurements of small molecules in surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS). In particular, they prepared homogeneous substrates of gold nanoparticle multilayers (AuNPs-ML) with hexagonal lattice by self-assembly technique. The optical and structural properties of substrates were characterized and glucose, sucrose and tryptophan were used for testing the performances of prepared substrates. The manuscript is well-organized and clearly written. Some points indicated below should be considered before the work can be accepted for publication in Nanomaterials Journal.

**Reply to the reviewer:**
We thank Reviewer 1 for her/his publication recommendation of our work. We have addressed all points indicated by the reviewer 2. We hope that the revised manuscript now is suitable for the publication in Nanomaterials.

**Comment:**
1) The Introduction has to be more focused on the specific argument of the manuscript, the number of cited references is certainly excessive. Please try to select those most relevant to the subject of the paper.

**Reply to the reviewer:**
We thank the suggestion from the reviewer 2. We have removed 7 references in our original manuscript to more focus on the preparation of high-performance sample substrate of gold nanoparticle multilayers for SALDI-MS.

**Comment:**
2) Please check the size of the hole cited in 2.3. Fabrication of Self-Assembled Monolayer of Gold Nanoparticles paragraph. It seems too large to me. In the same
paragraph, please better explained where the steel plate is initially positioned.

**Reply to the reviewer:**
The size of hole was 3 cm\(^2\) at the edge of the plastic cover.

After the evaporation of hexane and toluene for 24 hours, the steel plate was slowly immersed into the trough of glass petri dish. To transfer SAM of gold nanoparticles on the surface of deionized water, the steel plate was slowly pulled out of the sub-phase to transfer SAM of gold nanoparticles.

**Revision made:**
In the revised manuscript, we have added two sentences to describe the information of the initial position of steel plate. We wrote “To prepare sample substrate for SALDI-MS measurements, the steel plate (1 cm × 1 cm) was slowly immersed into the trough of glass petri dish. And then the steel plate was slowly pulled out of the sub-phase to transfer SAM of gold nanoparticles.”

**Comment:**
3) Please correct the details on the mass spectrometer at the beginning of 2.5 paragraph. Also, indicate the average laser power used during the experiments.

**Reply to the reviewer:**
We have provided the details of SALDI-MS measurements in the experimental section of “2.5. SALDI-MS Measurements”. The value of laser power has also been added in the experimental section.

**Revision made:**
We have provided the laser power used during the SALDI-MS experiments in the revised manuscript. We wrote “In the process of SALDI-MS detection, the laser power was tuned to be 130 μJ to acquire the best quality of mass spectra with high signal intensity, minimal interference and excellent signal/noise ratio.”

**Comment:**
4) As far as concerns the surface plasmon resonance absorption at ~ 520 nm, it doesn’t seem to be in agreement with the size of the calculated average size of gold nanoparticles (8.2 nm). A surface plasmonic resonance at around 520 nm is generally associated with larger particles. See for example Haiss et al Anal. Chem. 2007, 79,
4215-4221 and similar papers. The authors have to explain their result.

**Reply to the reviewer:**
Based on the results in the paper (Haiss et al. Anal. Chem. 2007, 79, 4215-4221), for sizes smaller than 25 nm the experimentally observed peak position is somewhat lower than predicted by theory. This may be attributed to the pronounced increase of the ratio of surface atoms to bulk atoms for particle diameters smaller than 20 nm. Our experimental result of size of gold nanoparticles corresponded to the data in work reported by Haiss et al.

**Comment:**
5) As far as concerns the spectra in Figure 3b, it is largely different from those reported in the Refs. 61 and 62 cited by the authors. What can be the reason for this?

**Reply to the reviewer:**
When the gold nanoparticles were self-assembled to form self-assembled monolayer (SAM) of gold nanoparticles, surface plasmon resonance absorption was revealed blue shift and the reflectance intensity of surface plasmon resonance was increased, indicating stronger nearfield interactions between nanoparticles. The difference between our work and Refs. 61 is the shape of nanocrystals. The difference between our work and Refs. 62 is the distance between each nanoparticle. But our work and Refs. 61 and 62 all showed plasmonic coupling.

**Comment:**
6) The authors affirm …Moreover, the mass spectrum of sample substrate of AuNPs-ML15 was measured to examine the background signals in SALDI-MS as shown in Figure S2. The background signals of AuNPs-ML15 also showed no significant glucose signal. If Figure S2 is related to the background signals, it is obvious that there no glucose signal. Please rewrite these sentences.

**Reply to the reviewer:**
We have rewritten the sentence to make it more clearly.

**Revision made:**
The sentence has been rewritten as “The background signals of AuNPs-ML15 also showed no significant signal.”
Comment:
7) It would be very useful if the authors add some comments about the specificity and the limit-of-detection that can be obtained by using the proposed substrates in SALDI-MS measurements. Also, a comparison with other not commercial substrates would be interesting for the reader.

Reply to the reviewer:
The limit-of-detection was 1 µM for the detection of glucose with the use of AuNPs-ML15 as the sample substrate in SALDI-MS.

The uses of sample substrate in SALDI-MS is just at the beginning stage. Therefore, there is no commercial sample substrates. However, the commercial matrixes of CHCA and DHB were investigated to compare the performance with our AuNPs-ML15 sample substrate. Overall, our sample substrate of AuNPs-ML15 exhibited the highest intensity of glucose signal, the lowest background noise and the excellent signal-to-noise ratio compared to that of commercial matrixes of CHCA and DHB.

Revision made:
We have added a sentence to reveal the limit-of-detection for glucose measurement with AuNPs-ML15 as the sample substrate in SALDI-MS. We wrote “Therefore, the limit-of-detection reached to 1 µM for the glucose measurement with the application of AuNPs-ML15 as the sample substrate in SALDI-MS.”

Comment:
8) Please check the manuscript for some misprints. In addition, a careful revision of the English language would be useful for eliminating some minor errors.

Reply to the reviewer:
We have checked and corrected all typos in the revised manuscript.