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Silicon nanostructures for sensing and bioimaging: General discussion

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**Silicon nanostructures for sensing and bioimaging: general discussion**

P. Ceroni, Y. Chao, C. Crucho, L. De Cola, A. Fucikova, A. Goyal, J. Joo, A. Reza Kamali, L. Osminkina, S. Silvestrini, H. Stephan, W. Sun, M. Lee Tang

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## Silicon nanostructures for sensing and bioimaging: general discussion

Paola Ceroni, Yimin Chao, Carina Crucho, Luisa De Cola, Anna Fucikova, Ankit Goyal, Jinmyoung Joo, Ali Reza Kamali, Liubov Osminkina, Simone Silvestrini, Holger Stephan, Wei Sun and Ming Lee Tang

**Holger Stephan** opened a general discussion of the paper by Jinmyoung Joo: (1) Have you determined the size and zeta potential of the SiNPs conjugate with the peptide RPARPAR? (2) Have you considered the formation of a protein corona around the SiNPs-RPARPAR conjugates?

**Jinmyoung Joo** replied: Yes, we have measured both the size and zeta potential of the RPARPAR conjugated SiNPs using DLS. The size is increased to ~20 nm after RPARPAR conjugation, and the zeta potential is ~+17 mV.

This [second point] is a very important point to note. The protein corona may be formed on the SiNP-RPARPAR conjugate and the degree of protein corona formation should be different according to the RPARPAR coverage. This also significantly affects the receptor binding followed by intracellular uptake. We are undertaking further study on this aspect.

**Anna Fucikova** asked: I would like to ask why the authors did just a 24 h study and why the article does not present any cytotoxicity testing. It is known that silicon nanoparticles with different surface passivation, and especially with different levels of surface oxidation, have dramatically different toxicological profiles. In a 48 h study the effect of inflammation and secondary toxicity could also be observed.

**Jinmyoung Joo** answered: We have studied the cellular uptake for 24 h post-incubation with SiNPs because the PL intensity becomes negligible as shown in Fig. 3 and 4 (DOI: 10.1039/c9fd00124g). In addition, we have carried out cytotoxicity tests with the SiNPs for 24 h, and found no obvious toxicity effects caused by silicon degradation. It is assumed that the current concentration range might be safe in use, thus not included in the paper. However, long-term monitoring especially focusing on toxicological profiles arising by inflammation and secondary effects should be a good suggestion. We will study this issue further separately as this manuscript is focussed on the multivalent interactions of SiNPs to the cell. We appreciate this question.

**Holger Stephan** opened a general discussion of the paper by Liubov A. Osminkina: In the experimental part you mentioned that the hydrodynamic diameter was determined by DLS. Which solvents did you use and are there differences in size distribution for AD-Si QDs and SCD-Si QDs?

**Liubov Osminkina** responded: The initial microparticle size after manufacture was quantified by laser diffraction (not DLS) using a Malvern Mastersizer 2000. Particle dispersion in water was aided by adding a drop of 5wt% Igepal surfactant. After further particle size reductions the size distributions of AD and SCD batches were not quantified.

**Ali Reza Kamali** opened a general discussion of the paper by Yimin Chao: Scheme 1 exhibits functionalised Si nanoparticles. The material was produced by the anodic polarization of boron-doped porous silicon in HF solutions, followed by an extensive chemical processing. In the absence of characterization methods such as XRD, how can the stability of the Si core in the nanoparticles be confirmed?

**Yimin Chao** replied: There is typical XRD data in the supporting information to confirm the nature of the Si core of the nanoparticles. In addition, the XPS data shown in Figure 2 in our article also proves the existence of the Si core.

**Ali Reza Kamali** asked: Please can you comment on the environmental impact associated with the preparation of functionalised Si nanoparticles?

**Yimin Chao** answered: The final product of functionalized Si nanoparticles has almost no impact on the environment. However, the preparation method in this work involves corrosive HF and other chemicals that should be managed with care during the synthesis. Some other bottom-up methods developed in my lab are environmentally friendly.

**Holger Stephan** remarked: The isothiocyanate group is quite reactive to amino groups of peptides and proteins. Can you make a statement about which cell (membrane) proteins bind to the ITC SiNPs and how long it takes to form a thiourea group in cell culture?

**Yimin Chao** responded: Apparently previous studies suggested that free ITCs penetrate cells by diffusion. Once inside the cells, they are rapidly conjugated *via* their -N=C=S group with intracellular thiols, mainly GSH.<sup>1-3</sup> There are no data on cell membrane proteins binding to ITC so far. In the case of our ITC-functionalised silicon nanopartides, we suppose that cellular uptake is mainly caused by endocytosis. Further studies will need to be performed to confirm which subtype of endocytosis occurs for our nanoparticles, whether it is clathrin-mediated, caveolae-dependent or another type.

1 Y. Zhang and E. C. Callaway, *Biochem. J.*, 2002, **364**, 301–307.

2 Y. Zhang, *Carcinogenesis*, 2012, **33**, 2–9.

3 Y. Zhang, *Carcinogenesis*, 2000, **21**, 1175–1182.

**Carina Crucho** queried: In Figure 1 in your paper, the peak at 2262 cm<sup>-1</sup> in the FTIR spectrum of allyl bromide capped SiNPs is mislabeled. Continuing with the

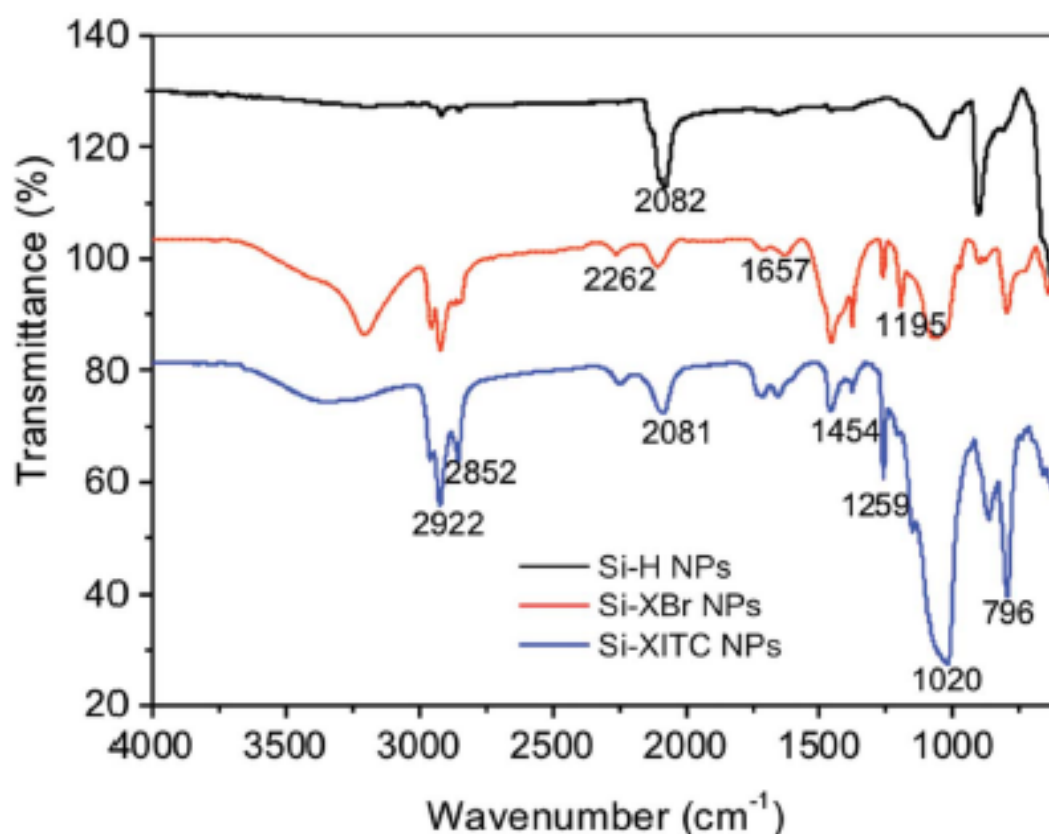


Fig. 1 FTIR spectra of H-terminated SiNPs, allyl bromide capped SiNPs, and ITC-capped SiNPs.

characterization, I wonder if the authors tried solution-state  $^1\text{H}$ -NMR to monitor the surface functionalization, as for example in Han Zuilhof's article in this *Faraday Discussion*.<sup>1</sup>

<sup>1</sup> DOI: 10.1039/c9fd00102f.

**Yimin Chao** replied: Thanks for pointing out this correction; the label  $2262\text{ cm}^{-1}$  has been shifted to the feature to the left; see Fig. 1 in the discussion.

NMR is an analysis tool at the molecular level that is not always feasible for nanoparticles. We did try NMR during the synthesis, but no nice data was obtained for this sample. We did obtain some NMR results for our other nanoparticles, *e.g.* amine-capped Si nanoparticles published in 2012.<sup>1</sup>

<sup>1</sup> J. H. Ahire, Q. Wang, P. R. Coxon, G. Malhotra, R. Brydson, R. Chen and Y. Chao, *ACS Appl. Mater. Interfaces*, 2012, 4, 3285–3292.

**Wei Sun** opened a general discussion of the paper by Luisa De Cola: The authors conclude that the product is a mixture of surface-decorated  $\text{SiO}_2$  nanoparticles and carbon QDs. Would the carbon QDs also contain a significant amount of Si? Prof. Yao He's article showed elemental mapping in which all particles seem to contain Si.<sup>1</sup> Personally I agree that the products should not be considered conventional Si(0) NPs, but they could still be called Si-containing NPs.



**Ming Lee Tang** addressed Luisa De Cola and Holger Stephan: Prof. De Cola – I have read and cited your other papers on triplets. What a coincidence to discover you're working on Si nanoparticles too! I really enjoyed reading the careful characterization of the nanoparticles in your article "Shedding light on the aqueous synthesis of silicon nanoparticles by reduction of silanes with citrates",<sup>1</sup> where you concluded based on all the evidence that they were not Si(0). However, in your article "Ultrasmall silicon nanoparticles as a promising platform for multimodal imaging",<sup>2</sup> a very similar synthesis (with a different reference) was used to create the Si-based scaffold, but no mention was made of it being silica in nature. Is there a chemical difference in the Si nanomaterial in both these papers?

1 DOI: 10.1039/c9fd00127a.

2 DOI: 10.1039/c9fd00091g.

**Holger Stephan** responded: In both articles<sup>1,2</sup> the synthesis route of He and coworkers was applied, namely the reaction of APTES and trisodium citrate in a microwave<sup>3,4</sup> (sample name MW-CS in the paper "Shedding light on the aqueous synthesis of silicon nanoparticles by reduction of silanes with citrates"<sup>1</sup>). Thus the reaction products in both papers are identical. They are silica nanoparticles with 3-aminopropyl-decorated surfaces mixed with thermal degradation products of citrate (carbon quantum dots).

1 DOI: 10.1039/c9fd00127a.

2 DOI: 10.1039/c9fd00091g.

3 Y. He, Y. Zhong, F. Peng, X. Wei, Y. Su, Y. Lu, S. Su, W. Gu, L. Liao and S.-T. Lee, *J. Am. Chem. Soc.*, 2011, **133**, 14192–14195, DOI: 10.1021/ja2048804.

4 Y. Zhong, F. Peng, F. Bao, S. Wang, X. Ji, L. Wang, Y. Su, S.-T. Lee and Y. He, *J. Am. Chem. Soc.*, 2013, **135**, 8350–8356, DOI: 10.1021/ja4026227.

**Simone Silvestrini** replied: We stand by the answer given by Prof. Holger. We add that it was indeed during the experimental activities leading to the publication of "Ultrasmall silicon nanoparticles as a promising platform for multimodal imaging" (DOI: 10.1039/c9fd00091g) that we realized a more in-depth study on the chemical nature of the particles was needed. We were particularly curious about the chemical mechanism that would lead citrate to reduce alkoxysilanes to elemental silicon, an aspect that in our opinion remains unclear and overlooked in the interpretation given by other authors.

**Paola Ceroni** asked Luisa De Cola: I appreciated the accurate and critical discussion in your article. My question is: which technique would you suggest to use as a fast and easy test for a new reaction of silicon nanocrystals to be sure of the nature of the nanoparticles?

**Simone Silvestrini** answered: An important lesson we learnt during this study that we would like to stress once more before answering the question is that we should trust no single test when we go to assess the merits of a new process to produce silicon nanoparticles. As is often the case in materials science, we should strive to validate new processes using a broad array of techniques, possibly going some lengths to get overlapping data from different approaches that support each other.

In the case of silicon(0) nanoparticles in the size range we are dealing with, X-ray photoelectron spectroscopy (XPS) has the merit of quick discrimination of silica from silicon, as discussed in our report and in the referenced material (ref. 11 and 12, in particular<sup>1,2</sup>). For this reason, we suggest its use to verify the chemical identity of Si(0) nanoparticles prepared *via* novel routes. It should be noted, however, that XPS will not yield structural information – *i.e.* on the crystallinity (X-ray powder diffraction) and the size distribution (X-ray- or light-scattering techniques) of the nanoparticles.

FT-IR is another useful tool in this sense, and one that is too often overlooked. While data interpretation for unknown samples can be more complex than in the case of XPS, FT-IR represents a great opportunity for the routine characterization of reaction products of established synthetic routes. This is especially true once reference samples become available for the target material and possibly for its pollutants/alternative products, which are likely to depend on the reaction itself.

1 M. L. Mastronardi, F. Maier-Flaig, D. Faulkner, E. J. Henderson, C. Kübel, U. Lemmer and G. A. Ozin, *Nano Lett.*, 2012, **12**, 337–342.

2 K. Sato, T. Izumi, M. Iwase, Y. Show, H. Morisaki, T. Yaguchi and T. Kamino, *Appl. Surf. Sci.*, 2003, **216**, 376–381.

**Carina Crucho** opened a general discussion of the paper by Holger Stephan: I wonder if the authors considered using solution-state <sup>1</sup>H-NMR to monitor the amine surface functionalization. Quantification by solution-state <sup>1</sup>H-NMR it is a method widely used to quantify organic moieties (hydrophobic and hydrophilic) attached to silica nanoparticles.<sup>1</sup> In the case of amine groups, from NMR spectra in D<sub>2</sub>O (as the protonated amines are soluble in water) it is possible to see the NMR peaks of the protons attached to the carbon next to the amine group or next to the silicon atom.

1 C. I. C. Crucho, C. Baleizão and J. P. S. Farinha, *Anal. Chem.*, 2017, **89**, 681–687, DOI: 10.1021/acs.analchem.6b03117.

**Holger Stephan** replied: Recent publications in *Analytical Chemistry*<sup>1,2</sup> describe suitable and reliable methods for the quantification of surface amino groups on silica nanoparticles (NPs) using solution NMR. These methods should in principle also be applicable for the determination of the amino groups of silicon NPs with 3-aminopropyl-decorated surfaces mixed with thermal degradation products of citrate.<sup>3</sup> However, we have not quantified the amino groups of silicon NPs by NMR. Here, a photometric assay (Kaiser Test) was employed to obtain information on the synthetically accessible surface amino groups. In a previous publication,<sup>4</sup> we used <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectroscopy to study the interactions of amino-functionalised silicon NPs with citric acid in D<sub>2</sub>O. By integration of the citric acid signals and comparison with the signals assigned to the silicon NPs, we found a 1/6 ratio of citrate compared to the 3-aminopropyl residue.

1 C. I. C. Crucho, C. Baleizão and J. P. S. Farinha, *Anal. Chem.* 2017, **89**(1), 681–687, DOI: 10.1021/acs.analchem.6b03117.

2 F. Kunc, V. Balhara, A. Brinkmann, Y. Sun, D. M. Leek and L. J. Johnston, *Anal. Chem.*, 2018, **90**, 13322–13330, DOI: 10.1021/acs.analchem.8b02803.

3 DOI: 10.1039/c9fd00127a.

4 N. Licciardello, S. Hunoldt, R. Bergmann, G. Singh, C. Mamat, A. Faramus, J. L. Z. Ddungu, S. Silvestrini, M. Maggini, L. De Cola and H. Stephan, *Nanoscale*, 2018, **10**, 9880–9891, DOI: 10.1039/c8nr01063c.



**Ankit Goyal** asked: This study of the bio-application of luminescent Si NPs is interesting. You mentioned in the discussion that the reason for biocompatibility and rapid renal clearance is the minimum surface modifications performed on the Si NP surface to achieve the imaging goals without hampering the size and surface charges of the particles. Could you please state whether similar behavior was found in every mouse you studied? It is highly surprising that NPs (even Si) do not accumulate in any other tissue or hamper any other metabolic activities. Did you check WBC and platelet counts after exposing the mice to NPs? Did you confirm the non-accumulation of Si NPs in renal and liver tissues by TEM? There are several metabolic pathways in mice (or other animals) that could quench luminescence, and inactive Si NPs could still accumulate in the system, so these studies could be decisive. Please provide your views on this.

**Holger Stephan** answered: The most reliable method to obtain exact and even quantitative data on the bio-distribution is positron emission tomography (PET) with radio-labelled compounds. With this technique, matrix effects can be excluded. In our previous paper<sup>1</sup> we showed that ultrasmall silicon particles are excreted very quickly, whereas the citrate-stabilized particles have the best pharmacokinetic properties. There is of course considerable activity at early time points (<1 h), in particular in the kidneys and liver. The same behaviour is found for the dual labelled (nuclear and fluorescence) silicon particles (5 nu/nu mice). We could further show that the particles remain intact, and most importantly, PET and optical imaging give the same results. We have not analysed certain organs/tissues/red and white cells in detail yet. However, we agree that this is of great interest. In particular, *ex vivo* audiographic images and fluorescence microscopy can provide detailed insight.

<sup>1</sup> N. Licciardello, S. Hunoldt, R. Bergmann, G. Singh, C. Mamat, A. Faramus, J. L. Z. Ddungu, S. Silvestrini, M. Maggini, L. De Cola and H. Stephan, *Nanoscale*, 2018, **10**, 9880–9891.