# Insights on Alanine and Arginine Binding to Silica with Atomic Resolution

Stefan Rauwolf, Saientan Bag, Rodrigo Rouqueiro, Sebastian Patrick Schwaminger, Ana Cristina Dias-Cabral, Sonja Berensmeier,* and Wolfgang Wenzel*

ABSTRACT: Interactions of biomolecules with inorganic oxide surfaces such as silica in aqueous solutions are of profound interest in various research fields, including chemistry, biotechnology, and medicine. While there is a general understanding of the dominating electrostatic interactions, the binding mechanism is still not fully understood. Here, chromatographic zonal elution and flow microcalorimetry experiments were combined with molecular dynamic simulations to describe the interaction of different capped amino acids with the silica surface. We demonstrate that ion pairing is the dominant electrostatic interaction. Surprisingly, the interaction strength is more dependent on the repulsive carboxy group than on the attracting amino group. These findings are essential for conducting experimental and simulative studies on amino acids when transferring the results to biomolecule−surface interactions.
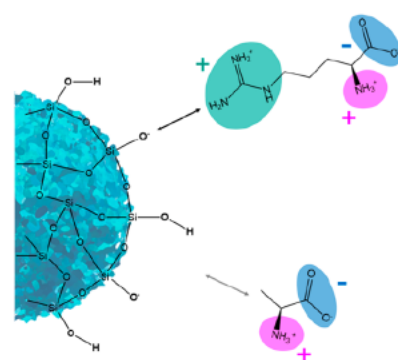
Amino acids (aa) are the building blocks for peptides and proteins, and understanding the mechanism that governs their interaction allows one to control the interactions of aa to different surfaces.[1−4] Controlling these interactions is essential in several research fields in chemistry, medicine, and biotechnology.[5−13] Inorganic materials, especially silica, play an important role in fields where the interaction of aa with the surface is important, such as chromatography,[14] biosensors,[15−17] and drug delivery.[18−24] Additionally, these interactions play a role in the origin of life, because, in its early stages, peptides were built by condensation of aa on inorganic solid surfaces such as silica.[25−27] Silica features two types of surface groups responsible for the intrinsic surface properties and the resulting interactions with other molecules: siloxane bridges (Si−O−Si) and silanol groups (Si−OH). Silanol groups deprotonate at pH > 3, leading to a negative charge density on the surface which increases with pH.[28,29] The silica surface and its features regarding biomolecule interactions have been discussed thoroughly in various reviews.[30−32]

Due to the broad interest to different scientific fields, experimental and theoretical studies identified electrostatic interactions as the driving force for interaction of silica with aa,[33−35] peptides,[36−38] and proteins.[39−42] However, the influences of the individual groups of aa on these interactions are still not fully understood.[29,43] The pH influences the charge of molecules and surfaces and, therefore, electrostatic interactions. Amino acids are primarily zwitterionic at ambient conditions due to α amino and α carboxy groups' charges.[44] Only aa such as histidine, lysine, and arginine carry a positive charge. Glutamic acid and aspartic acid bear an overall negative charge, each. These five aa carry the charge of proteins and thus strongly influence protein interactions.

The aim of this study is to elucidate, with atomic precision, which functional groups of aa contribute to the binding and whether electrostatic interactions are solely or dominantly responsible for binding. To obtain insight about the influence of individual aa groups on their adsorption to the silica surface, the interaction of selected aa and their specific capped variants with silica were analyzed. For the first time, N and C capped variations of amino acids are used for experimental interaction studies with silica surfaces. In earlier studies we were able to show that in aqueous systems only basic positively charged amino acids adsorb with silica.[45,46] Therefore, in this study only the zwitterionic L alanine (Ala) and the positively charged L arginine (Arg) are investigated. These model aa were chosen on the basis of their backbone and functional group charge. The respective aa variants with blocked N and/or C terminus were acetylated L alanine (Ac A), ethylated L alanine (A ethyl), *tert* butylated L alanine (A tbutyl), L arginine (Arg), acetylated L arginine (Ac R), methylated L arginine (R OMe), and a double capped L arginine (Ac R OMe).

In the case of Ala when the negative carboxy group is blocked, an overall positive charge due to the remaining amino
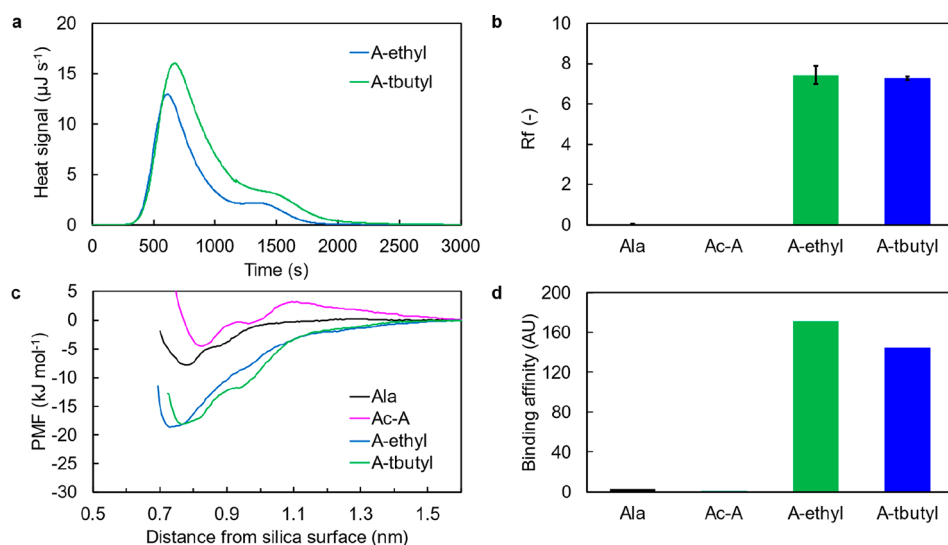
Figure 1. (a) Heat exchange profile of 10 mM L alanine ethyl (A ethyl) ester and 10 mM L alanine *tert* butyl ester (A tbutyl) obtained from FMC experiments. Injection loop, 30 μL; mobile phase: 1.5 mL h^{-1} H$_2$O, pH 7.4. (b) Retention factors (R_f) of alanine variants (50 mM) calculated from measured retention time in relation to a nonbinding tracer (1 g L^{-1} uracil) using zonal elution experiments. Injection, 20 μL; mobile phase, 2 mL min^{-1} of 10 mM TRIS, pH 7.4. Error bars indicate standard deviation resulting from three runs per experiment. (c) PMF profile for the interaction between different capped and noncapped alanines to the silica surface as obtained from umbrella sampling simulation. (d) Binding affinity of different alanine species calculated by integrating (see eq S1) the PMF curves.
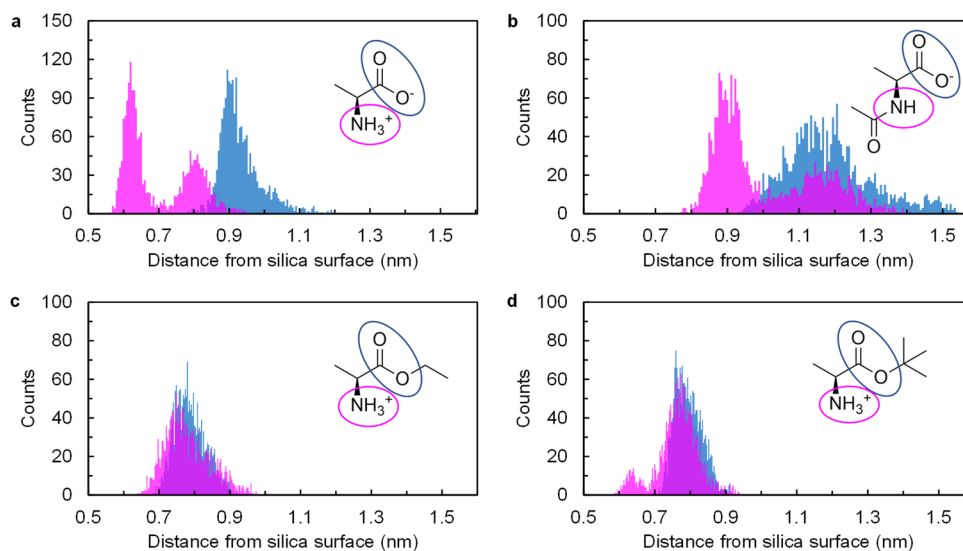

Figure 2. Histograms of the distances of the C and N termini of L alanine (a), acetyl L alanine (b), L alanine ethyl ester (c), and L alanine *tert* butyl ester (d) from the silica surface when the aa are adsorbed on silica as obtained from the MD simulation. In magenta and blue are the amino and carboxy groups of the backbone, respectively. For more details on the "distances" calculated here, see Figure S4 of the Supporting Information and text therein.

group should be observed resulting in binding to silica. For Arg, depending on the blocked groups, different strengths of interaction are expected.

To facilitate the elucidation of the interaction mechanism between aa and a silica surface, thermodynamic studies by means of flow microcalorimetry (FMC) were used to in situ monitor the enthalpy of the interactions. Compared to other microcalorimetric techniques, FMC can be used to simulate a packed bed chromatographic system at microscale. FMC can dissect the subprocesses involved in the interaction between molecules and surface and, as a consequence, be used to discriminate between different energy contributions.[47−49] The FMC used in this study can detect power changes with a

magnitude of 10^{-7} W, resulting in an energy resolution in the order of 10^{-9} J, enabling analysis of very weak interactions.[47−49] The FMC is ideally complemented with zonal elution chromatography (ZE) to provide fundamental data for molecular dynamic (MD) simulation.[45] For MD simulation the atomistic model of silica and corresponding force field parameters from a database by Emami et al. was used.[28] To investigate the adsorption behavior, the Q3 silica surface model (see Supporting Information Figure S1 and text therein for more details) was chosen. It considers 4.7 silanol groups per nm^2 of surface, of which 14% are deprotonated at a pH of 7.4. For the experiments all aa variants were purchased and designed for simulation (Figure S2 and Figure S3).
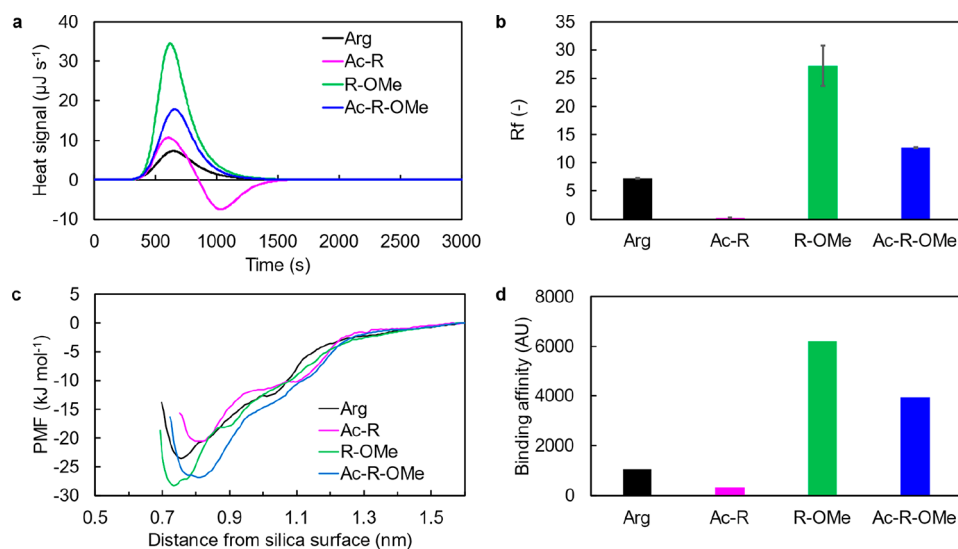
Figure 3. (a) Heat exchange profile of different capped arginine (10 mM) obtained from FMC experiments. Injection loop, 30 μL; mobile phase, 1.5 mL h^{-1} H$_2$O, pH 7.4. (b) Retention factors (R_f) of arginine variants (50 mM) calculated from measured retention time in relation to a nonbinding tracer (1 g L^{-1} uracil) using zonal elution experiments. Injection, 20 μL; mobile phase, 2 mL min^{-1} of 10 mM TRIS, pH 7.4. Error bars indicate standard deviation resulting from three runs per experiment. (c) PMF profile for the interaction between different capped and noncapped arginines (Args) to the silica surface as obtained from umbrella sampling simulation. (d) Binding affinity of different Arg species calculated by integrating (see eq S1) the PMF curves.

Different capped alanines were investigated for the influence of the backbone amino and carboxy group inherited in every aa. For the zwitterionic Ala, there was no detectable heat signal in FMC, suggesting no interaction between alanine and silica. This is supported by ZE experiments in which Ala elutes at the same time as the tracer (Figure 1B, Table S1). This behavior can be explained by the MD data where the distances of the centers of mass of the different alanine side groups from the silica surface were calculated via simulation. For more details on the "distances" calculated here, see Figure S4 of the Supporting Information and text therein. For noncapped Ala, the amino group can be near the surface, while the carboxy group is pendent (Figure 2A). Two peaks for the amino group at 0.6 and 0.8 nm indicate different conformations. The amino group of Ala can interact via electrostatic interactions or H bonds with the water on the surface.[50] Solid state NMR investigation suggests that the interaction of L alanine with hydrated silica most likely happens with water (mobile phase) molecules on the surface, resulting in washing out of the silica in a dynamic setup.[50] Ben Shir et al. found an N−Si distance of 0.4−0.42 nm for L alanine and glycine on silica and declared it as direct binding as no molecule such as water would fit between the molecules.[51−53] The potentials of mean force (PMF, Figure 1C) profiles were calculated by umbrella sampling (US). By integrating the PMF profiles, the binding affinity was calculated showing no binding affinity (Figure 1D).

For the Ac A derivative FMC also showed a small exothermic peak, which can be associated with salt effects on the silica surface due to pH adjustment (Figure S5). Therefore, silica and Ac A do not interact. The MD data support the assumption of no binding: the integration of the PMF profiles showed no binding affinity for Ac A with silica (Figure 1C,D). In the case of Ac A, the amino group is blocked, and the aa bears a total net charge of −1. As can be seen in the histogram, both groups are far from the surface with very broad distributions, indicating no relevance for the adsorption to silica (Figure 2B). However, ZE experiments indicate electro

static repulsion of Ac A from the silica surface. The negative charge of the aa results in faster run times through the column than the tracer solution. This observation points out the mitigating effect of the negatively charged carboxy group on the interaction of aa and silica (Table S1).

For both carboxy capped derivatives A ethyl and A tbutyl only exothermic peaks with net heats of −6.2 ± 0.21 and −8.9 ± 0.31 mJ, respectively, were observed in the FMC (Figure 1A, Table S2), indicating adsorption to silica, as the occurrence of interactions of an exothermal nature contributes majorly to the adsorptive process enthalpy.[49] The interaction event is supported by the ZE experiments, which show a retention factor of >7 (Figure 1B, Table S1). In chromatography, a retention factor of 1 means a slight interaction with the column. A retention factor of 20 means strong interactions because the analyte is spending a lot of time interacting with the resin. Retention factors > 20 are problematic because it means extremely long run times and poor sensitivity due to peak broadening.[54] The retention can be explained by the overall net charge of +1 for both aa. Integration of the PMF profiles from simulation further validated the interaction (Figure 1C,D). A second exothermic peak for A ethyl with 1.0 ± 0.32 mJ and for A tbutyl with 0.78 ± 0.29 mJ overlapping the binding peak indicates the rearrangement of both aa derivatives following the binding process. The signal is aligned at a time beyond aa pulse residence time at the FMC cell (around 480 s after heat signal start), compatible with the establishment of a favorable arrangement of the adsorbed aa at the surface. The energy for this rearrangement would be given by the decrease of enthalpy from the first to the second observed exothermic event.[49] This behavior can be explained by steric hindrance by the capping groups (Figure S6 and Figure S7). However, this hindrance does not affect the retention time in chromatography significantly and therefore has only a little to no effect on the interaction between aa and silica.
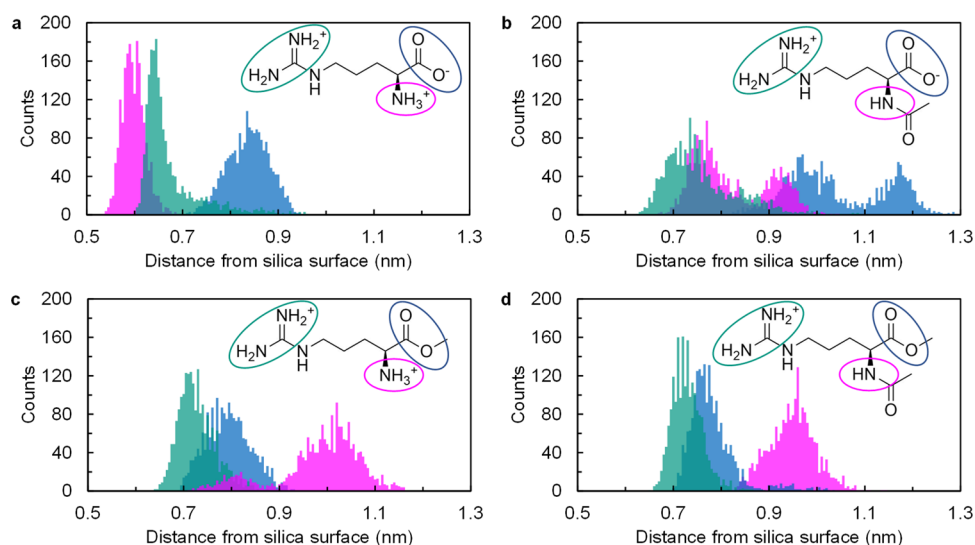
Figure 4. Histograms of the distances of the C and N termini of L arginine (a), acetyl L arginine (b), L arginine methyl ester (c), and acetyl L arginine methyl ester (d) from the silica surface when the aa are adsorbed on silica as obtained from the MD simulation. In magenta and blue are the amino and carboxy groups of the backbone, respectively. In turquoise is the guanidine side group. For more details on the "distances" calculated here, see Figure S4 of the Supporting Information and text therein.

The most interesting effect the FMC shows is the missing of an initial endothermic peak. According to literature, the FMC profile characteristic for ion exchange involves a first endothermic peak related to the desolvation entropic process overlapped with an exothermic peak related to the electrostatic interaction itself. The missing of the endothermic peak indicates a reduced contribution from desolvation subprocess to adsorption, essential to an ion exchange binding mechanism, and suggests ion pairing as a possible mechanism.[36]

MD simulations indicate a contribution of the methylated carboxy group as well as the amino group to the interaction with silica surfaces. The interaction contribution is indicated by two superimposed histograms at around 0.75−0.80 nm (Figure 2C,D). Furthermore, ZE experiments with 200 mM sorbitol as H bond competitor and 1 M NaCl revealed the electrostatic nature of the binding. While there was no influence on the retention using sorbitol, NaCl negated the interaction of A ethyl and A tbutyl (Figure S8). Snapshots of the simulations illustrate the spatial location of the different alanine derivatives to silica (Figure S9). Both the simulation and ZE experiments show the same trend for binding affinities: A ethyl ∼ A tbutyl > Ala > Ac A.

FMC and ZE experiments combined with MD simulation elucidated the influence of the aa C and N termini on the interaction of Ala. Given these findings, Arg with a strongly positive guanidinium side group was investigated for the additional interaction effects of the functional side group in combination with the backbone groups.[35,36,55]

In FMC the tendency R OMe > Ac R OMe > Arg > Ac R for binding enthalpy was observed (Table S3). The same trend was found in ZE experiments and MD simulation in terms of affinity (Figure 3). Considering that electrostatic interactions are the dominating forces for interaction, the charges of the aa can explain the trend. R OMe bears a net charge of +2 and has the highest affinity. As shown for Ala, the amino group of the backbone can interact with the surface in addition to the guanidine side chain. The net heats of the single exothermic event for the binding of Arg and R OMe are −2.81 ± 0.08 and −10.31 ± 0.92 mJ (Figure 3B, Table S3), respectively. Both

the ZE experiments with $R_{f,\text{R-OMe}} = 27 \pm 3.3$ being four times that of $R_{f,\text{Arg}} = 7.25 \pm 0.06$ and integration of PMF profiles from simulation support the findings of the FMC. In the case of noncapped Arg, MD shows both positively charged amino and guanidine groups are at distances of 0.60 and 0.65 nm, respectively, from the silica surface during adsorption, whereas the negatively charged carboxy group is pendent (Figure 4A). MD indicates direct binding of the guanidine group to the surface.[51−53] For R OMe, when the carboxy group is capped, all three functional groups can be near the surface (Figure 4C and Figure S10). The positive amino and guanidine groups can interact at distances of 0.8 and 0.7 nm from the surface, respectively, while the capped carboxy group could interact through H bonds between the carbonyl group (C=O) and surface silanol groups.[35,37,38]

In contrast, when the amino group is capped and the zwitterionic Ac R is used, ZE experiments indicate a low affinity; a significantly lower R_f of 0.29 ± 0.00 was measured compared to Arg (Figure 3B, Table S4). Ac R has an overall neutral charge due to its zwitterionic state and low affinity comparable to Ala, and other zwitterionic aa are expected.[45,55,56] Low affinity is supported by the FMC signal; the exothermic event (binding) with −5.0 ± 0.14 mJ and the endothermic event (more consistent with elution) with 4.3 ± 0.20 mJ are about the same size, resulting in a net heat of −0.74 ± 0.09 mJ (Table S3 and Figure S11). Further proof can be found in the MD simulations. For Ac R the broad peaks and the greater distances of all groups to the surface indicate very loose binding. The histogram for Ac R indicates that the adsorption is mainly mediated by the guanidine side group of the Ac R and the negative carboxy group is pendent (Figure 4B). This result is clear evidence for the interaction of amino acids with silica through amine groups. The fact that in FMC for Ac R binding and elution could be observed with a negative total neat heat and the R_f is still somewhat higher than for the comparable zwitterionic Ala indicates the interaction of the guanidine group with silica is stronger than the α amino group of the aa backbone with silica. These observations demonstrate again the influence of the negatively charged carboxy group on

the adsorption of basic aa. The results for the doubly capped Ac R OMe confirmed this influence. The derivative shows in FMC a higher interaction heat of -5.9 ± 0.36 mJ compared to that of the noncapped Arg, despite having the same net charge of $+1$. Due to the missing negative charge on the carboxy group the doubly capped aa experiences no repulsion from the surface, resulting in stronger interaction. However, it also shows a lower binding enthalpy than R OMe, because it lacks the additional positively charged amino group. The same trend is found in ZE experiments. This effect can be explained by MD, where the side chain guanidine (0.7 nm) and the carbonyl group (0.8 nm) are near the surface, while the capped amino group is pendent (Figure 4D and Figure S10); only the guanidine group is interacting with silica, while the carboxy group cannot mitigate the interaction due to the capping group. More detailed analysis concluding the closest distance of the different functional groups (of uncapped and capped Arg) from the silica surface are presented in the Supporting Information (see Figure S10 and text therein).

The thermogram profiles for arginine derivatives (Figure 3A) show considerable differences compared to alanine (Figure 1A). No second exothermic peak is observed for the first, indicating the absence of rearrangements during interaction. This reinforces the idea of arginine multipoint attachment to silica surface through positively charged amino and guanidine groups, which is not so prone to rearrangement processes.

Competitive ZE experiments with sorbitol and NaCl with arginine derivatives (Figure S8) showed the same trend as that for alanine, indicating that only electrostatic interactions play a role for binding. The positively charged groups are attracted, while negatively charged groups are repelled, from the negatively charged silica surface. This indicates electrostatic interactions mediate the adsorption between positively charged amino and guanidine groups with deprotonated silanol groups on the surface.[36,38,55,57] Snapshots of the simulations illustrate the binding of arginine to silica (Figure S12).

Besides the influence of the functional groups on binding of amino acids to silica the FMC suggests the same mechanism for binding of the Arg derivatives as for Ala. The results indicate again that the binding mechanism is not accompanied by an ion exchange. Since the aa thermograms miss the initial endothermic peak, the binding mechanism probably follows the principle of ion pairing between positively charged amino and guanidine groups with siloxide groups (SiO^-) as already theorized for peptides.[36]

In summary, this study shows how flow microcalorimetry experiments combined with chromatographic zonal elution experiments and molecular dynamic simulation can reveal the specific influence of different functional groups on the binding affinity of aa to silica in aqueous environments. Investigating different capped Ala and Arg showed the overall charge dominating the strength of interaction with silica and exposed the influence of the negatively charged carboxy group due to repulsion from the negatively charged silica surface. Furthermore, this is the first study which experimentally proves that aa binding on silica does not follow ion exchange but an ion pairing mechanisms. These results help to improve models and to further understand the binding behaviors for amino acid, peptide, and protein adsorption not only to silica but also other oxide surfaces. These findings can be applied in various research fields, ranging from purification of biomolecules to drug delivery systems.

AUTHOR INFORMATION

Corresponding Authors

Sonja Berensmeier — *Department Mechanical Engineering, Bioseparation Engineering Group, Technical University of Munich, 85748 Garching, Germany;* orcid.org/0000 0002 4943 848X; Email: s.berensmeier@tum.de

Wolfgang Wenzel — *Institute for Nanotechnology, Karlsruhe Institute of Technology, 76344 Eggenstein Leopoldshafen, Germany;* orcid.org/0000 0001 9487 4689; Email: wolfgang.wenzel@kit.edu

Authors

Stefan Rauwolf — *Department Mechanical Engineering, Bioseparation Engineering Group, Technical University of Munich, 85748 Garching, Germany;* orcid.org/0000 0002 0756 2749

Saientan Bag — *Institute for Nanotechnology, Karlsruhe Institute of Technology, 76344 Eggenstein Leopoldshafen, Germany;* orcid.org/0000 0003 1000 7719

Rodrigo Rouqueiro — *Department of Chemistry, CICS UBI Health Science Research Center, University Beira Interior, 6200 506 Covilhã, Portugal*

Sebastian Patrick Schwaminger — *Department Mechanical Engineering, Bioseparation Engineering Group, Technical University of Munich, 85748 Garching, Germany;* Present Address: Department of Chemical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139, USA; orcid.org/0000 0002 8627 0807

Ana Cristina Dias Cabral — *Department of Chemistry, CICS UBI Health Science Research Center, University Beira Interior, 6200 506 Covilhã, Portugal;* orcid.org/0000 0002 2759 7762

ABBREVIATIONS

Ac A	acetylated L alanine
Ac R	acetylated L arginine
Ac R OMe	acetyl L arginine methyl ester
A ethyl	ethylated L alanine
Ala,	L alanine
Arg	L arginine
A tbutyl	*tert* butylated L alanine
FF	force field
FMC	flow microcalorimetry
MD	molecular dynamics
PMF	potential mean force
R OMe	methylated L arginine
ZE	zonal elution.

REFERENCES

(1) Sheldon, R. A.; van Pelt, S. Enzyme immobilisation in biocatalysis: Why, what and how. *Chem. Soc. Rev.* 2013, 42, 6223–6235.

(2) Costa, D.; Savio, L.; Pradier, C. M. Adsorption of amino acids and peptides on metal and oxide surfaces in water environment: A synthetic and prospective review. *J. Phys. Chem. B* 2016, 120, 7039–7052.

(3) Das, P.; Reches, M. Review insights into the interactions of amino acids and peptides with inorganic materials using single molecule force spectroscopy. *Biopolymers* 2015, 104, 480–494.

(4) Kang, J. H.; McCusker, L. B.; Deem, M. W.; Baerlocher, C.; Davis, M. E. Further investigations of racemic and chiral molecular sieves of the STW topology. *Chem. Mater.* 2021, 33, 1752–1759.

(5) Henderson, C. J.; Pumford, E.; Seevaratnam, D. J.; Daly, R.; Hall, E. A. H. Gene to diagnostic: Self immobilizing protein for silica microparticle biosensor, modelled with sarcosine oxidase. *Biomaterials* 2019, 193, 58–70.

(6) Govrin, R.; Schlesinger, I.; Tcherner, S.; Sivan, U. Regulation of surface charge by biological osmolytes. *J. Am. Chem. Soc.* 2017, 139, 15013–15021.

(7) Yuan, P.; Mao, X.; Wu, X.; Liew, S. S.; Li, L.; Yao, S. Q. Mitochondria targeting, intracellular delivery of native proteins using biodegradable silica nanoparticles. *Angew. Chem., Int. Ed.* 2019, 58, 7657–7661.

(8) Pyles, H.; Zhang, S.; De Yoreo, J. J.; Baker, D. Controlling protein assembly on inorganic crystals through designed protein interfaces. *Nature* 2019, 571, 251–256.

(9) El Shetehy, M.; Moradi, A.; Maceroni, M.; Reinhardt, D.; Petri Fink, A.; Rothen Rutishauser, B.; Mauch, F.; Schwab, F. Silica nanoparticles enhance disease resistance in Arabidopsis plants. *Nat. Nanotechnol.* 2021, 16, 344–353.

(10) Matange, K.; Tuck, J. M.; Keung, A. J. DNA stability: a central design consideration for DNA data storage systems. *Nat. Commun.* 2021, 12, 1358.

(11) Benmerzoug, S.; Rose, S.; Bounab, B.; Gosset, D.; Duneau, L.; Chenuet, P.; Mollet, L.; Le Bert, M.; Lambers, C.; Geleff, S.; Roth, M.; Fauconnier, L.; Sedda, D.; Carvalho, C.; Perche, O.; Laurenceau, D.; Ryffel, B.; Apetoh, L.; Kiziltunc, A.; Uslu, H.; Albez, F. S.; Akgun, M.; Togbe, D.; Quesniaux, V. F. J. STING dependent sensing of self DNA drives silica induced lung inflammation. *Nat. Commun.* 2018, 9, 5226.

(12) Nguyen, M. K.; Nguyen, V. H.; Natarajan, A. K.; Huang, Y.; Ryssy, J.; Shen, B.; Kuzyk, A. Ultrathin silica coating of DNA origami nanostructures. *Chem. Mater.* 2020, 32, 6657–6665.

(13) Lee, J. Y.; Kim, M. K.; Nguyen, T. L.; Kim, J. Hollow Mesoporous Silica Nanoparticles with Extra Large Mesopores for Enhanced Cancer Vaccine. *ACS Appl. Mater. Interfaces* 2020, 12, 34658–34666.

(14) González García, T.; Margola, T.; Silvagni, A.; Mancin, F.; Rastrelli, F. Chromatographic NMR spectroscopy with hollow silica spheres. *Angew. Chem., Int. Ed.* 2016, 55, 2733–2737.

(15) Yang, X.; Qiu, P.; Yang, J.; Fan, Y.; Wang, L.; Jiang, W.; Cheng, X.; Deng, Y.; Luo, W. Mesoporous materials based electrochemical biosensors from enzymatic to nonenzymatic. *Small* 2021, 17, No. 1904022.

(16) Su, Q.; Xu, P.; Zhou, L.; Wu, F.; Dong, A.; Wan, Y.; Qian, W. Real Time and Label Free Monitoring of Biomolecular Interactions within Complex Biological Media Using a Silica Colloidal Crystal Film. *ACS Appl. Mater. Interfaces* 2020, 12, 35950–35957.

(17) Yuan, P.; Mao, X.; Liew, S. S.; Wu, S.; Huang, Y.; Li, L.; Yao, S. Q. Versatile multiplex endogenous RNA detection with simultaneous signal normalization using mesoporous silica nanoquenchers. *ACS Appl. Mater. Interfaces* 2020, 12, 57695–57709.

(18) Hofmann, C.; Duerkop, A.; Baeumner, A. J. Nanocontainers for analytical applications. *Angew. Chem., Int. Ed.* 2019, 58, 12840–12860.

(19) Xuan, M.; Shao, J.; Zhao, J.; Li, Q.; Dai, L.; Li, J. Magnetic mesoporous silica nanoparticles cloaked by red blood cell membranes: Applications in cancer therapy. *Angew. Chem., Int. Ed.* 2018, 57, 6049–6053.

(20) Yang, G.; Liu, Y.; Wang, H.; Wilson, R.; Hui, Y.; Yu, L.; Wibowo, D.; Zhang, C.; Whittaker, A. K.; Middelberg, A. P. J.; Zhao, C. X. Bioinspired core shell nanoparticles for hydrophobic drug delivery. *Angew. Chem., Int. Ed.* 2019, 58, 14357–14364.

(21) Yuan, P.; Zhang, H.; Qian, L.; Mao, X.; Du, S.; Yu, C.; Peng, B.; Yao, S. Q. Intracellular delivery of functional native antibodies under hypoxic conditions by using a biodegradable silica nanoquencher. *Angew. Chem., Int. Ed.* 2017, 56, 12481–12485.

(22) Hong, X.; Zhong, X.; Du, G.; Hou, Y.; Zhang, Y.; Zhang, Z.; Gong, T.; Zhang, L.; Sun, X. The pore size of mesoporous silica nanoparticles regulates their antigen delivery efficiency. *Sci. Adv.* 2020, 6, No. eaaz4462.

(23) Yan, M.; Xie, L.; Tang, J.; Liang, K.; Mei, Y.; Kong, B. Recent advances in heterosilica based micro/nanomotors: designs, biomed ical applications, and future perspectives. *Chem. Mater.* 2021, 33, 3022–3046.

(24) Juneja, R.; Vadarevu, H.; Halman, J.; Tarannum, M.; Rackley, L.; Dobbs, J.; Marquez, J.; Chandler, M.; Afonin, K.; Vivero Escoto, J. L. Combination of Nucleic Acid and Mesoporous Silica Nano particles: Optimization and Therapeutic Performance In Vitro. *ACS Appl. Mater. Interfaces* 2020, 12, 38873–38886.

(25) Erastova, V.; Degiacomi, M. T.; Fraser, D. G.; Greenwell, H. C. Mineral surface chemistry control for origin of prebiotic peptides. *Nat. Commun.* 2017, 8, 2033.

(26) Navrotsky, A.; Hervig, R.; Lyons, J.; Seo, D. K.; Shock, E.; Voskanyan, A. Cooperative formation of porous silica and peptides on the prebiotic Earth. *Proc. Natl. Acad. Sci. U. S. A.* 2021, 118, e2021117118.

(27) Guo, W.; Kinghorn, A. B.; Zhang, Y.; Li, Q.; Poonam, A. D.; Tanner, J. A.; Shum, H. C. Non associative phase separation in an evaporating droplet as a model for prebiotic compartmentalization. *Nat. Commun.* 2021, 12, 3194.

(28) Emami, F. S.; Puddu, V.; Berry, R. J.; Varshney, V.; Patwardhan, S. V.; Perry, C. C.; Heinz, H. Force field and a surface model database for silica to simulate interfacial properties in atomic resolution. *Chem. Mater.* 2014, 26, 2647–2658.

(29) Buszewska Forajta, M.; Markuszewski, M. J.; Kaliszan, R. Free silanols and ionic liquids as their suppressors in liquid chromatog raphy. *J. Chromatogr. A* 2018, 1559, 17–43.

(30) Rimola, A.; Costa, D.; Sodupe, M.; Lambert, J. F.; Ugliengo, P. Silica surface features and their role in the adsorption of biomolecules: computational modeling and experiments. *Chem. Rev.* 2013, 113, 4216–4313.

(31) Lambert, J. F. Adsorption and polymerization of amino acids on mineral surfaces: a review. *Origins Life Evol. Biospheres* **2008**, *38*, 211−242.

(32) Fenoglio, I.; Fubini, B.; Ghibaudi, E. M.; Turci, F. Multiple aspects of the interaction of biomacromolecules with inorganic surfaces. *Adv. Drug Delivery Rev.* **2011**, *63*, 1186−1209.

(33) Holinga, G. J.; York, R. L.; Onorato, R. M.; Thompson, C. M.; Webb, N. E.; Yoon, A. P.; Somorjai, G. A. An SFG study of interfacial amino acids at the hydrophilic SiO2 and hydrophobic deuterated polystyrene surfaces. *J. Am. Chem. Soc.* **2011**, *133*, 6243−6253.

(34) Razvag, Y.; Gutkin, V.; Reches, M. Probing the interaction of individual amino acids with inorganic surfaces using atomic force spectroscopy. *Langmuir* **2013**, *29*, 10102−10109.

(35) Rimola, A.; Sodupe, M.; Ugliengo, P. Affinity scale for the interaction of amino acids with silica surfaces. *J. Phys. Chem. C* **2009**, *113*, 5741−5750.

(36) Patwardhan, S. V.; Emami, F. S.; Berry, R. J.; Jones, S. E.; Naik, R. R.; Deschaume, O.; Heinz, H.; Perry, C. C. Chemistry of aqueous silica nanoparticle surfaces and the mechanism of selective peptide adsorption. *J. Am. Chem. Soc.* **2012**, *134*, 6244−6256.

(37) Emami, F. S.; Puddu, V.; Berry, R. J.; Varshney, V.; Patwardhan, S. V.; Perry, C. C.; Heinz, H. Prediction of specific biomolecule adsorption on silica surfaces as a function of pH and particle size. *Chem. Mater.* **2014**, *26*, 5725−5734.

(38) Maity, S.; Zanuy, D.; Razvag, Y.; Das, P.; Alemán, C.; Reches, M. Elucidating the mechanism of interaction between peptides and inorganic surfaces. *Phys. Chem. Chem. Phys.* **2015**, *17*, 15305−15315.

(39) Hoehne, M.; Samuel, F.; Dong, A.; Wurth, C.; Mahler, H. C.; Carpenter, J. F.; Randolph, T. W. Adsorption of monoclonal antibodies to glass microparticles. *J. Pharm. Sci.* **2011**, *100*, 123−132.

(40) Tarasevich, Y. I.; Monakhova, L. I. Interaction between globular proteins and silica surface. *Colloid J.* **2002**, *64*, 482−487.

(41) Turci, F.; Ghibaudi, E.; Colonna, M.; Boscolo, B.; Fenoglio, I.; Fubini, B. An integrated approach to the study of the interaction between proteins and nanoparticles. *Langmuir* **2010**, *26*, 8336−8346.

(42) Mohammad Beigi, H.; Hayashi, Y.; Zeuthen, C. M.; Eskandari, H.; Scavenius, C.; Juul Madsen, K.; Vorup Jensen, T.; Enghild, J. J.; Sutherland, D. S. Mapping and identification of soft corona proteins at nanoparticles and their impact on cellular association. *Nat. Commun.* **2020**, *11*, 4535.

(43) Puddu, V.; Perry, C. C. Peptide adsorption on silica nanoparticles: evidence of hydrophobic interactions. *ACS Nano* **2012**, *6*, 6356−6363.

(44) Nelson, D. L.; Cox, M. M. *Lehninger principles of biochemistry*, 6th ed.; W. H. Freeman: New York, 2013.

(45) Bag, S.; Rauwolf, S.; Suyetin, M.; Schwaminger, S. P.; Wenzel, W.; Berensmeier, S. Buffer influence on the amino acid silica interaction. *ChemPhysChem* **2020**, *21*, 2347−2356.

(46) Bag, S.; Rauwolf, S.; Schwaminger, S. P.; Wenzel, W.; Berensmeier, S. DNA binding to the silica: Cooperative adsorption in action. *Langmuir* **2021**, *37*, 5902−5908.

(47) Rosa, S. A.S.L.; da Silva, C. L.; Aires Barros, M. R.; Dias Cabral, A. C.; Azevedo, A. M. Thermodynamics of the adsorption of monoclonal antibodies in phenylboronate chromatography: Affinity versus multimodal interactions. *J. Chromatogr. A* **2018**, *1569*, 118−127.

(48) Silva, G. L.; Marques, F. S.; Thrash, M. E.; Dias Cabral, A. C. Enthalpy contributions to adsorption of highly charged lysozyme onto a cation exchanger under linear and overloaded conditions. *J. Chromatogr. A* **2014**, *1352*, 46−54.

(49) Da Silva, G. F. L.; Plewka, J.; Tscheließnig, R.; Lichtenegger, H.; Jungbauer, A.; Dias Cabral, A. C. M. Antibody Binding Heterogeneity of Protein A Resins. *Biotechnol. J.* **2019**, *14*, No. 1800632.

(50) Guo, C.; Holland, G. P. Alanine adsorption and thermal condensation at the interface of fumed silica nanoparticles: A solid state NMR investigation. *J. Phys. Chem. C* **2015**, *119*, 25663−25672.

(51) Ben Shir, I.; Kababya, S.; Amitay Rosen, T.; Balazs, Y. S.; Schmidt, A. Molecular level characterization of the inorganic bioorganic interface by solid state NMR: alanine on a silica surface, a case study. *J. Phys. Chem. B* **2010**, *114*, 5989−5996.

(52) Ben Shir, I.; Kababya, S.; Schmidt, A. Binding specificity of amino acids to amorphous silica surfaces: Solid state NMR of glycine on SBA 15. *J. Phys. Chem. C* **2012**, *116*, 9691−9702.

(53) Ben Shir, I.; Kababya, S.; Schmidt, A. Molecular details of amorphous silica surfaces determine binding specificity to small amino acids. *J. Phys. Chem. C* **2014**, *118*, 7901−7909.

(54) Ornaf, R. M., Dong, M. W. Key concepts of HPLC in pharmaceutical analysis. In *Handbook of Pharmaceutical Analysis by HPLC*; Elsevier, 2005; Vol. 6, pp 19−45. DOI: 10.1016/S0149 6395(05)80046 7.

(55) Vlasova, N. N.; Golovkova, L. P. The adsorption of amino acids on the surface of highly dispersed silica. *Colloid J.* **2004**, *66*, 657−662.

(56) Basiuk, V. A.; Gromovoy, T. Y. Free energies of amino acid adsorption on silica in neutral aqueous medium as estimated from high performance liquid chromatographic retention data. *Amino Acids* **1994**, *7*, 305−309.

(57) Gao, Q.; Xu, W.; Xu, Y.; Wu, D.; Sun, Y.; Deng, F.; Shen, W. Amino acid adsorption on mesoporous materials: influence of types of amino acids, modification of mesoporous materials, and solution conditions. *J. Phys. Chem. B* **2008**, *112*, 2261−2267.