Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Immobilization approaches can affect protein dynamics: a surface-enhanced infrared spectroscopic study on lipid-protein interactions

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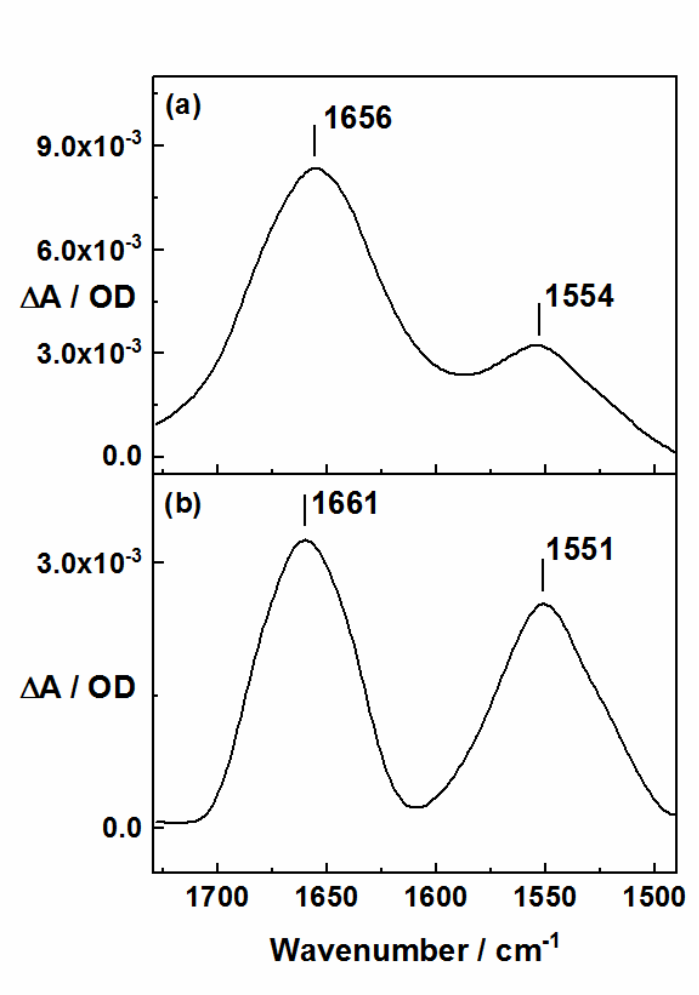

Figure S1. SEIDA spectra of N-terminal immobilized αS on a SAM of mixed NHS-PEG-SH : MT(PEG)$_4$ with different linker-spacer ratios. (a) NHS-PEG-SH : MT(PEG)$_4$ 1:1. (b) NHS-PEG-SH : MT(PEG)$_4$ 1:100. The amide I band intensity is ≈ 3x higher for the 1:1 linker : spacer composition, thus a higher number of available binding sites increases the protein concentration at the surface, but not in a linear manner. The spectra were recorded 2 hours after αS reaction with the modified mixed SAMs and subsequent rinsing with H_2O.

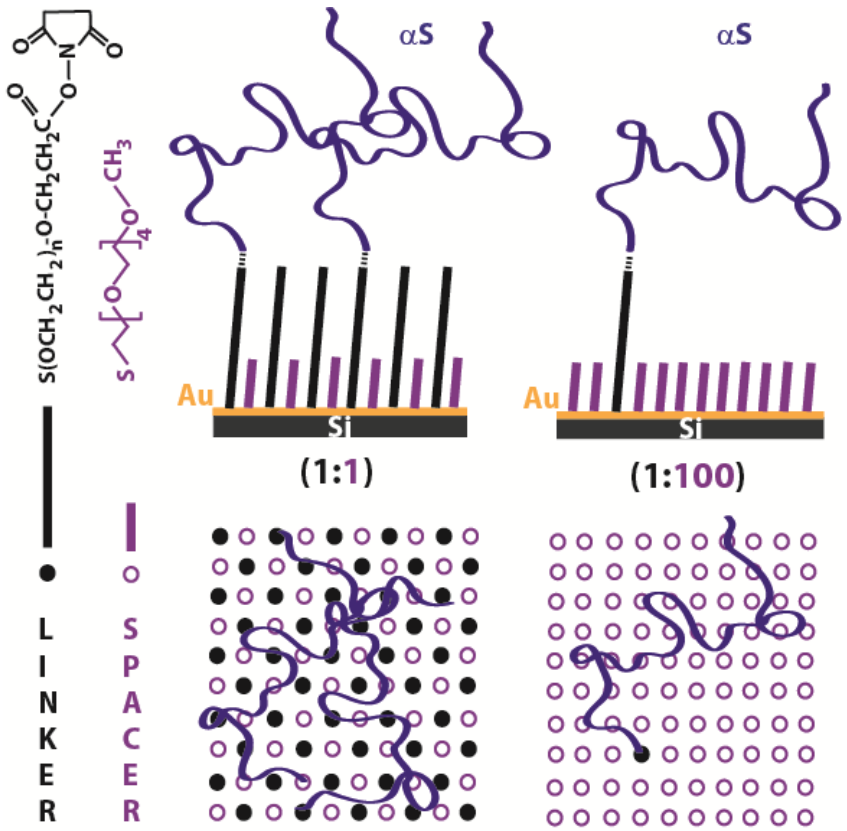

Figure S2. Schematic sketch of αS immobilization on mixed NHS-PEG-SH : MT(PEG)$_4$ SAMs with different linker : spacer compositions (1:1 and 1:100). The NHS-PEG-SH linker molecules are drawn in black (black bars and solid black circles). The MT(PEG)$_4$ spacers are shown in violet (violet bars, open violet circles). For a linker : spacer composition of 1:1, the comparatively long sequence length of αS monomers covers a large number of linker molecule. Since each monomer binds to only one single linker molecule, the rest of the available linkers are probably simply blocked by the protein. Therefore, not every linker molecule can react with αS molecules due to the high surface coverage by the immobilized protein. For a linker : spacer composition 1:100, fewer linker molecules are available for protein immobilization. In addition, the lower number of immobilized αS monomers leads to less protein-protein interactions which should be prevented in this study.

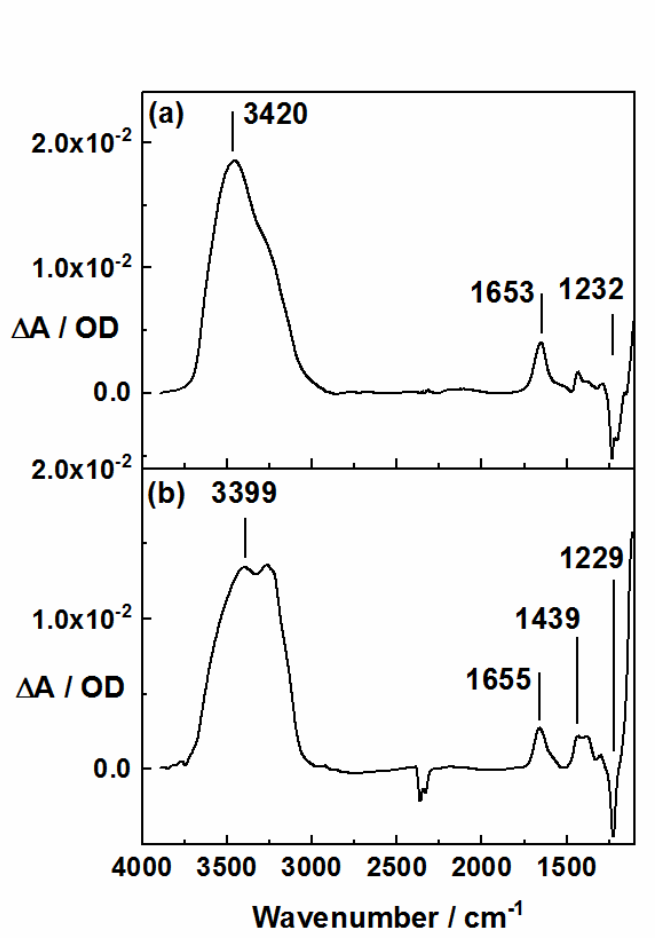

Figure S3. Modification of the SAM with (a) αS C-terminal antibody. The SEIDA spectrum shows covalent binding and subsequent immobilization of αS C-terminal antibody to the PEG SAM by conversion of NHS intermediate esters to amide bonds. The negative band at ≈ 1230 cm^{-1} reveals NHS cleavage during antibody reaction with the SAM. Note that the antibody concentration is low and the antibody amide I band is overlaid by the stronger water band. **(b) ANTA.** The SAM was modified with ANTA, and the formation of the NTA-Ni^{2+} complex was monitored. The negative band at 1229 cm^{-1} reveals reaction of SAM NHS intermediates with ANTA. IR bands at ≈ 1440 cm^{-1} can be assigned to the asymmetric carboxylate stretch frequency of the ANTA-Ni^{2+} complex. H_2O bands occur at ≈ 3400 cm^{-1} and ≈ 1655 cm^{-1}.

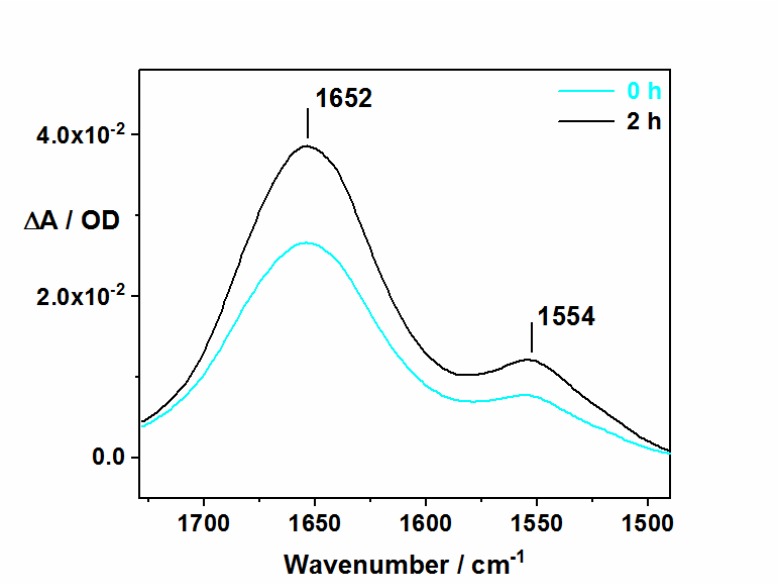

Figure S4. C-terminal immobilization of αS to the NHS-PEG-SH:MT(PEG)$_4$ 1:100 SAM functionalized with αS C-terminal antibody. SEIDA spectra show successful immobilization after 2 hours of reaction time. The amide I band at 1652 cm^{-1} indicates predominant disordered structure of the immobilized αS.

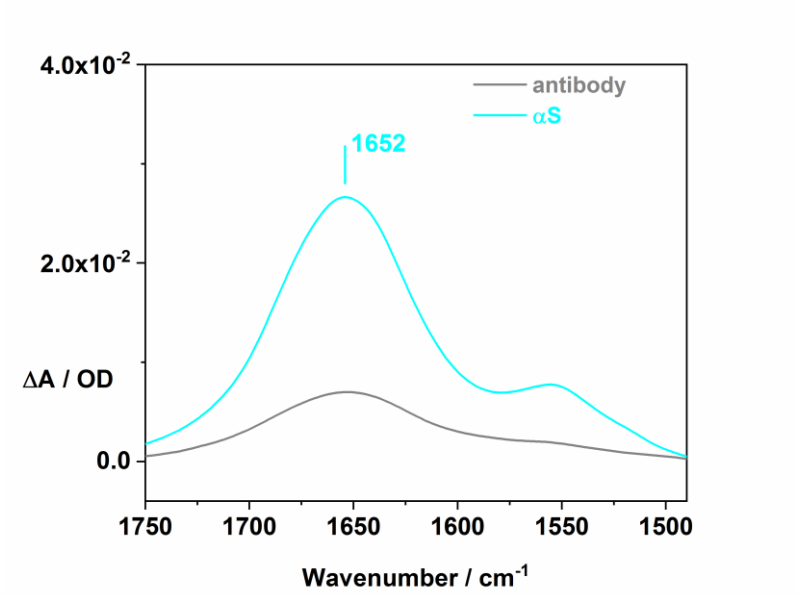

Figure S5. Contribution of the antibody absorbance to the total amide I signal of C-terminally immobilized αS. The absorbance of the covalently bound αS antibody is significantly lower and does not interfere the detection of conformational changes of immobilized αS. Although the surface was rigorously rinsed after protein immobilization, we cannot completely exclude the occurence of physisorbed αS.

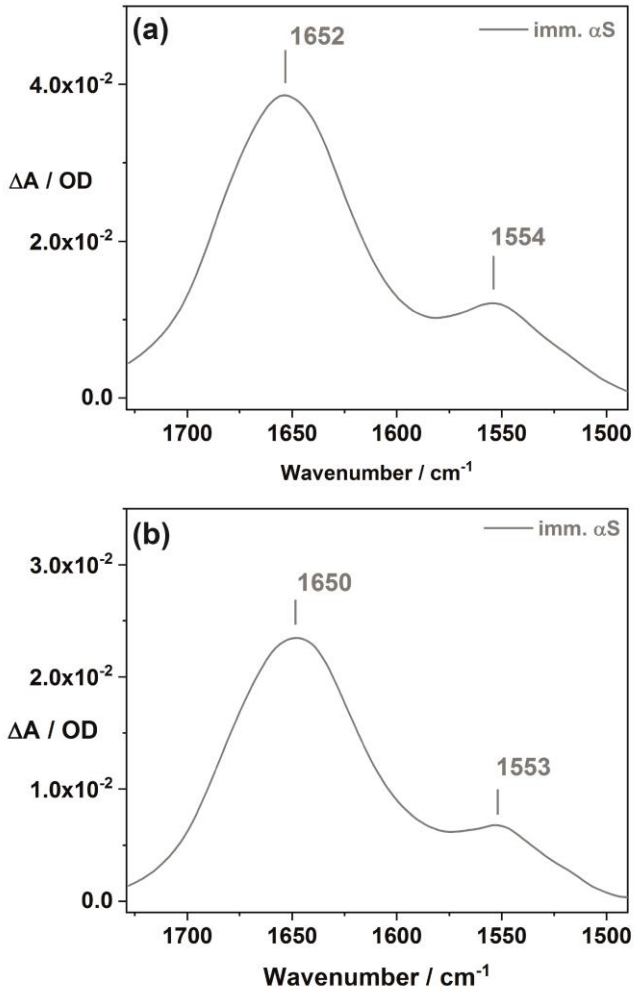

Figure S6. SEIDA spectra of C-terminally immobilized αS (a) before addition of POPG SUVs to the supernatant solution and (b) before addition of supplementary αS to the supernatant solution. The spectra belong to the same experiments shown in Figure 4 in the main text.

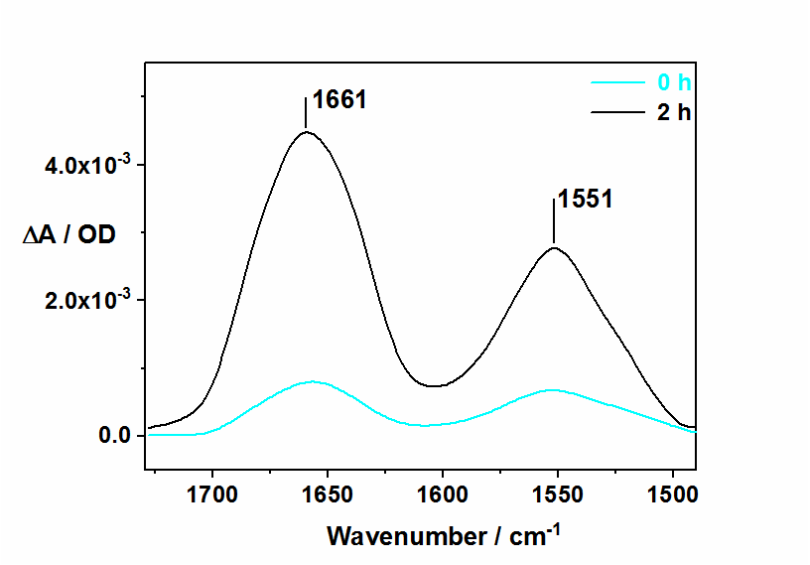

Figure S7. N-terminal immobilization of αS to the Ni^{2+}-NTA modified NHS-PEG-SH : $MT(PEG)_4$ 1:100 SAM. The reaction time was 2 hours. The amide I band at 1661 cm^{-1} indicates that αS adopts a more α-helical structure after immobilization via the N-terminus.

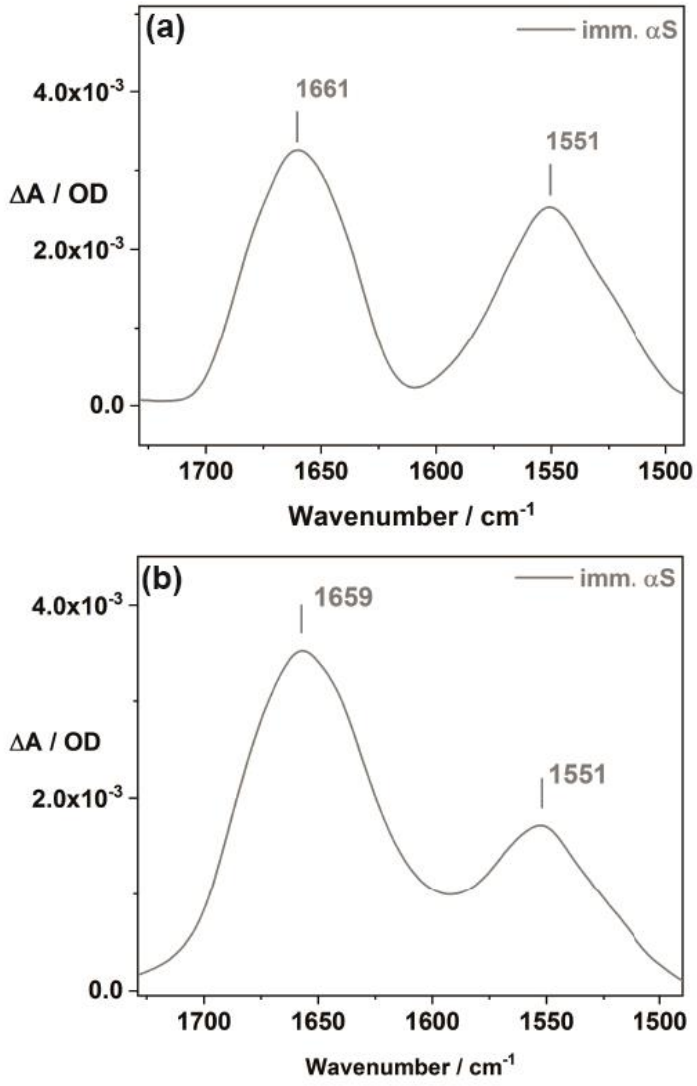

Figure S8. SEIDA spectra of N-terminally immobilized αS (a) before addition of POPG SUVs to the supernatant solution and (b) before addition of supplementary αS to the supernatant solution. The spectra belong to the same experiments shown in Figure 5 in the main text.