Supplementary materials for

# ATR-FTIR biosensors for antibody detection

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**Figure S1: Grafting of 11-mercaptoundecanoic acid**

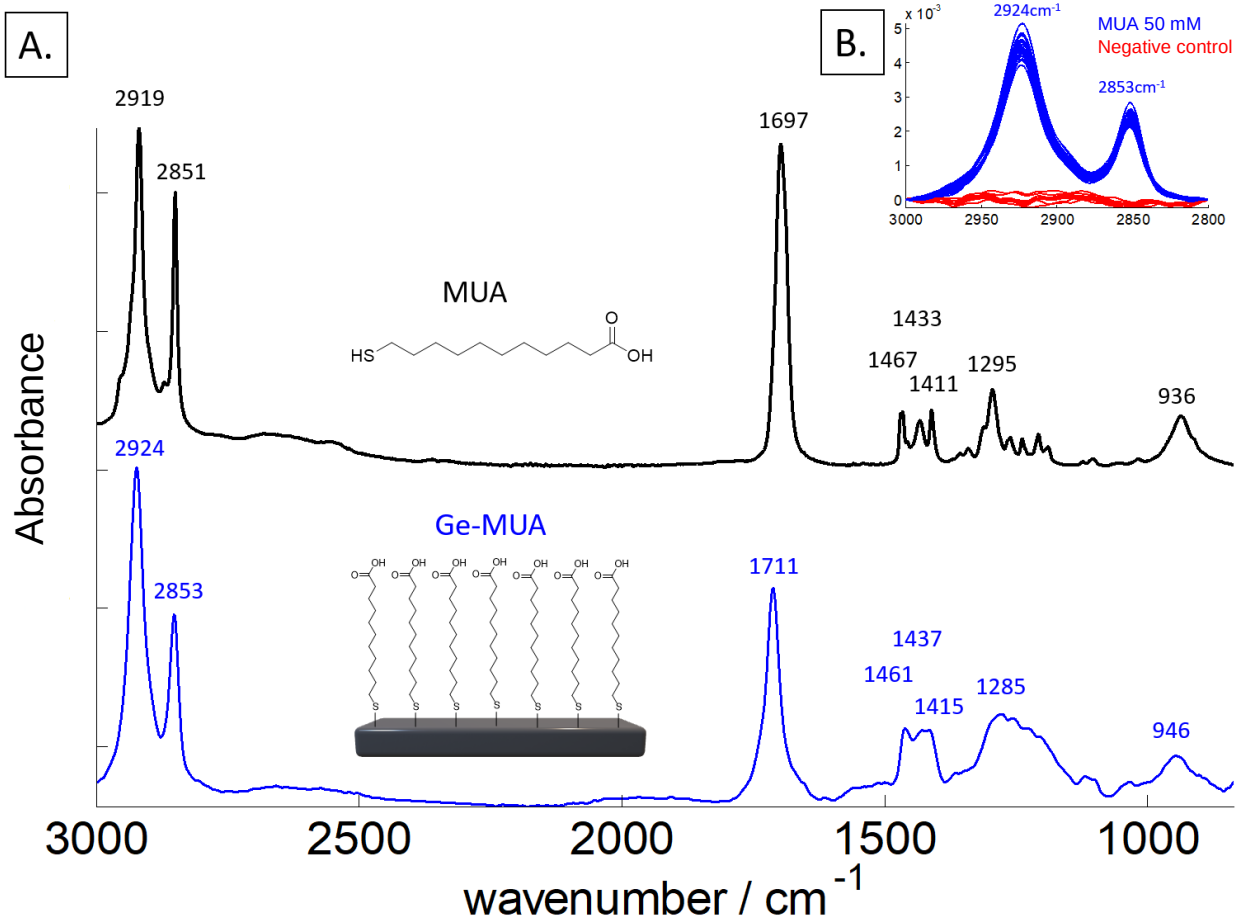


Figure S1: A. Ge oxide removal and 11-mercaptoundecanoic acid (MUA) SAM functionalization using 1:1 IPA-water 50mM thiol solution heated at 60°C (blue line). The FTIR spectrum of the 11-mercaptoundecanoic acid grafted onto the surface is characterized by three well-defined bands: $\nu_{as}(CH_2)$ at 2924cm$^{-1}$, $\nu_s(CH_2)$ at 2853cm$^{-1}$ and $\nu$(C=O) at 1711 cm$^{-1}$. The black line is the spectrum of pure MUA in IPA dried onto the diamond crystal. Spectra have been vertically offset for the sake of the clarity. B. Ge crystals immerged in IPA without MUA (negative control) show no specific bands. The inset reports the results in the 3000-2800 cm$^{-1}$ spectral range for 20 repetitions for MUA binding and 10 repetitions for the control.

**Figure S2: Grafting of deuterated $d_{25}$ dodecanethiol**

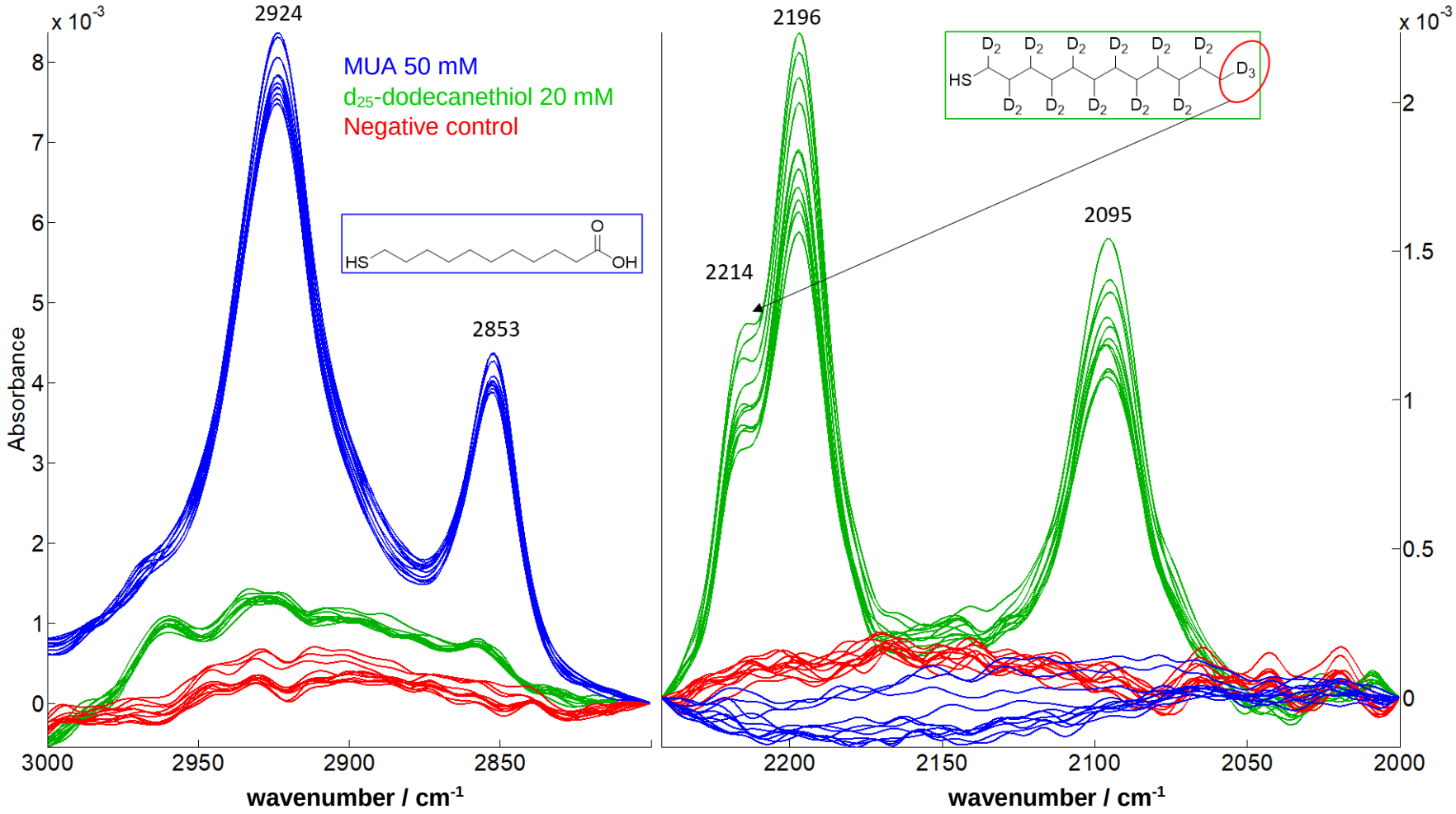


Figure S2: Grafting of 50mM MUA (blue line), 20 mM deuterated dodecanethiol (green line) and control (red line). The grafting of fully deuterated MUA (green line) is characterized by the bands $\nu_{as}$ ($C^2H_2$) at 2196 $cm^{-1}$, $\nu_s$ ($C^2H_2$) at 2095 $cm^{-1}$ and $\nu_{as}$($C^2H_3$) at 2214 $cm^{-1}$. Ten independent spectra are reported for each condition.

**Figure S3: NHS ester formation**

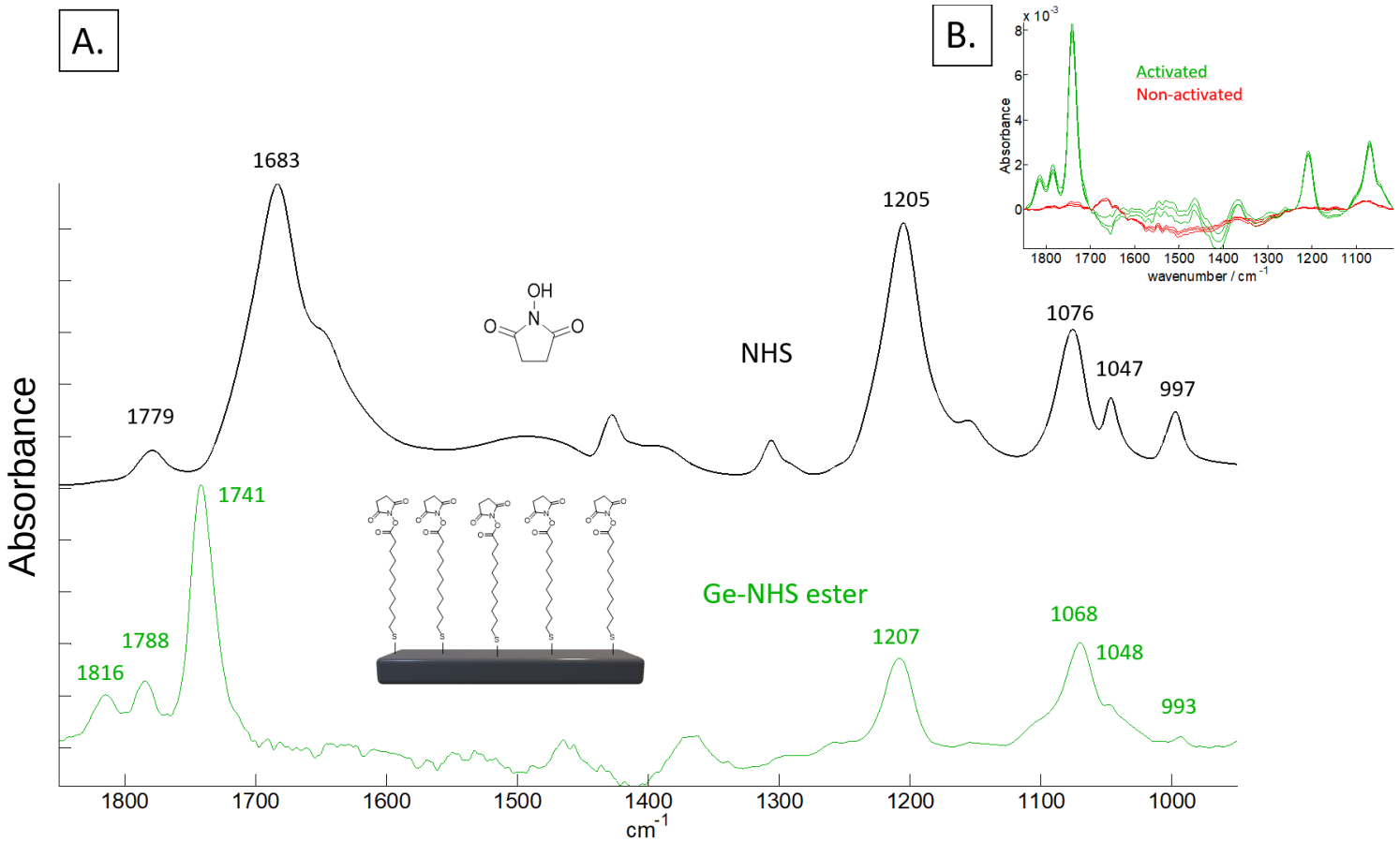


Figure S3: A. Activation of the carboxyl group of MUA using EDC/NHS. The black line is the spectrum of pure NHS in water dried onto the diamond IRE. The green line is the carboxyl of Ge grafted-MUA transformed into an NHS ester function. The band at 1683 $cm^{-1}$ of the black line corresponds to the vibrations of the ketone's groups of the NHS while the band at 1741 $cm^{-1}$ of the green line corresponds to the vibration of the ester bond. Two additional well-defined bands of the ester bond are observable at 1816 $cm^{-1}$ and 1788 $cm^{-1}$ and two bands specific to NHS are present at 1207 $cm^{-1}$ and 1068 $cm^{-1}$. Spectra have been vertically offset for the sake of the clarity. B. Comparison of FTIR spectra of activated and non-activated MUA. For non-activated MUA, the Ge grafted-MUA crystals were immerged in water without EDC/NHS (negative control). Spectra are presented for 4 independent experiments.

**Figure S4: BSA immobilization**

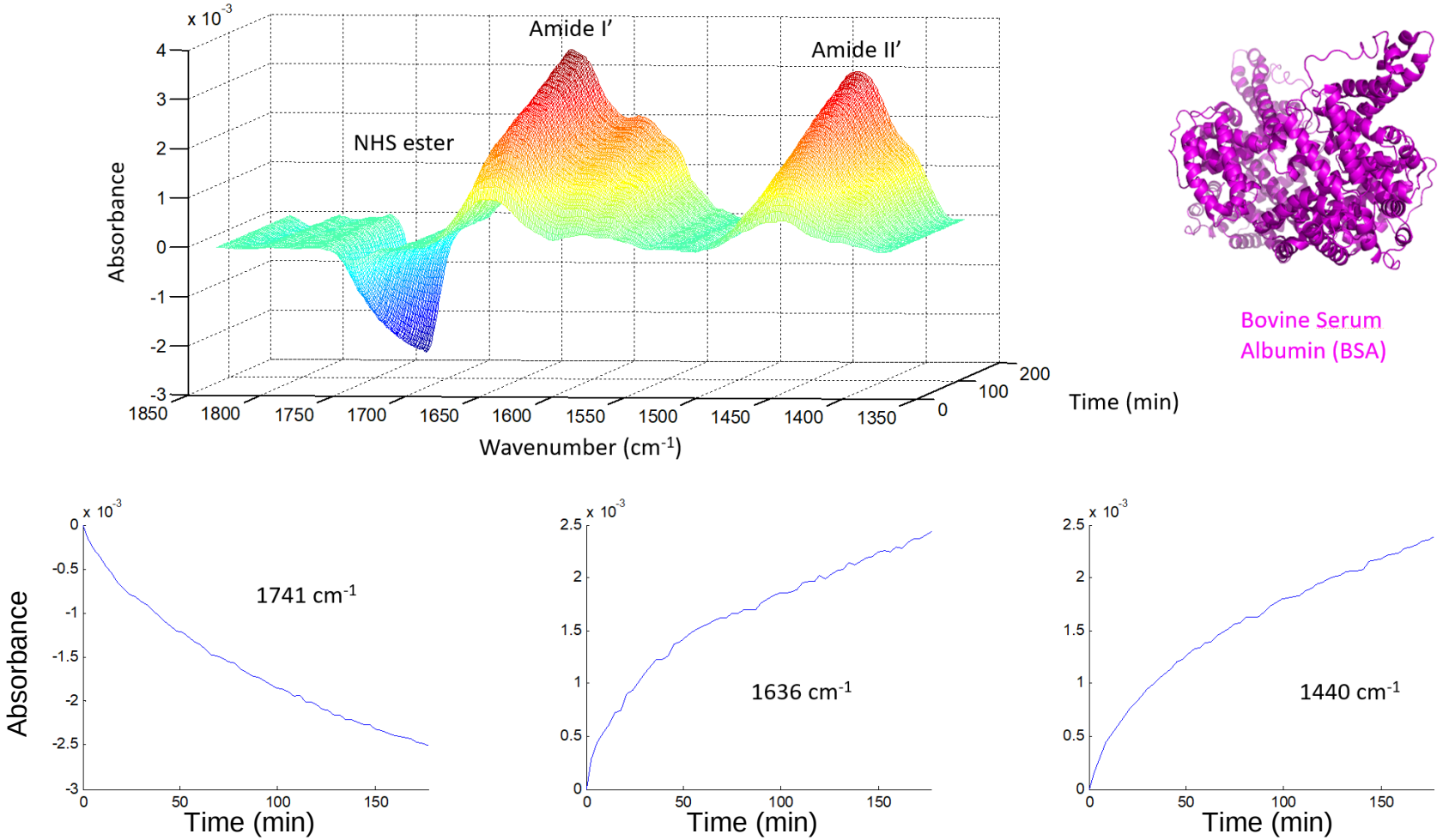


Figure S4: Grafting kinetics of bovine serum albumin on the activated germanium surface monitored by ATR-FTIR spectroscopy. The color gradient indicates the absorbance intensity. As the NHS ester band at 1741 $cm^{-1}$ disappears (in blue), amide bands at 1636 $cm^{-1}$ and 1440 $cm^{-1}$ appear (in red) over time. Duration of the kinetics is 3 hours, and a spectrum is recorded every 3 min (60 spectra in total). Evolution of the absorbance of the three specific wavenumbers over time (min) is shown below.

**Figure S5: BSA immobilization kinetic correlation**

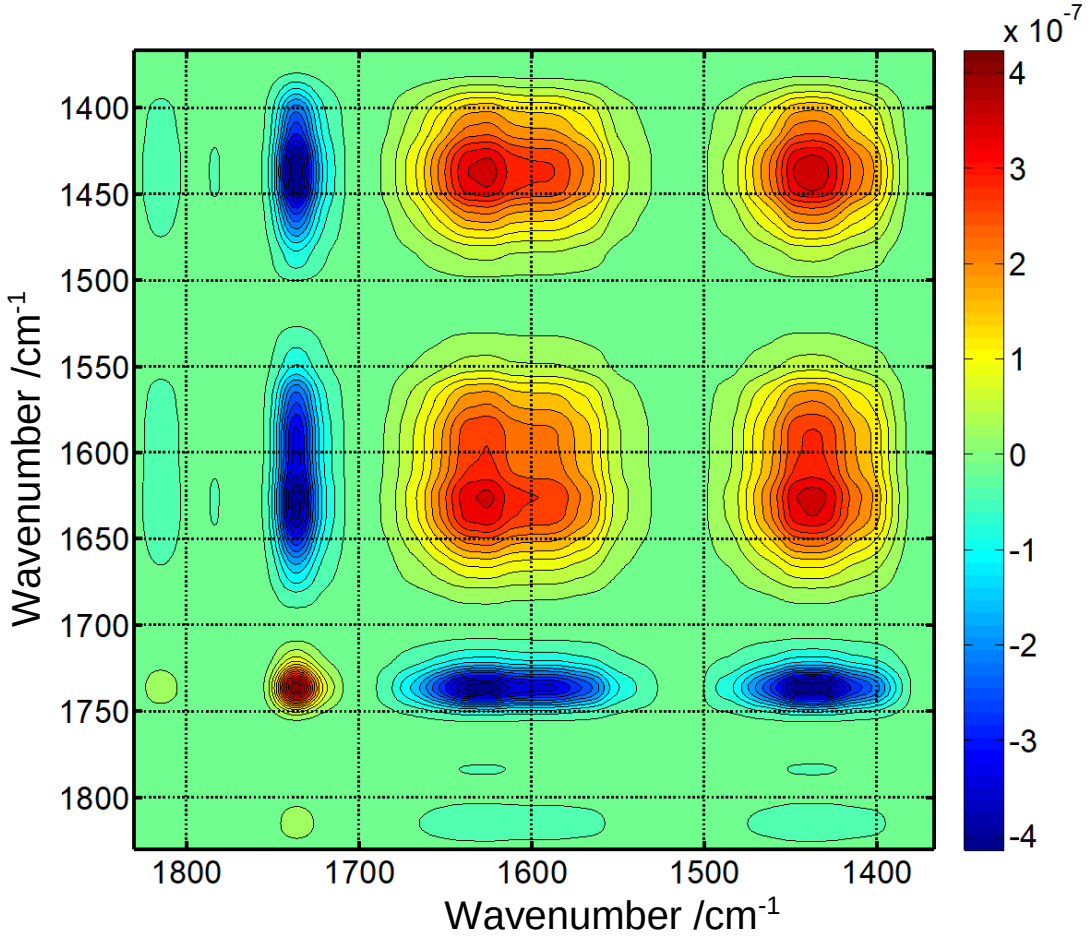


Figure S5: Magnitude of the covariance between the different wavenumbers during the grafting of BSA. The disappearance of the NHS ester at 1741cm$^{-1}$ is significantly and negatively correlated with the appearance of the amide bands at 1636 cm$^{-1}$ and 1640 cm$^{-1}$. Correlation coefficient between 1741 and 1636: -0.993, corresponding p-value: 3.3 $10^{-55}$. Correlation coefficient between 1741 and 1440 cm$^{-1}$: -0.998, corresponding p-value: 4.96 $10^{-72}$. Correlation coefficient between 1636 and 1440 cm$^{-1}$: +0.997, corresponding p-value: 3.610$^{-68}$.

**Figure S6: Avidin immobilization**

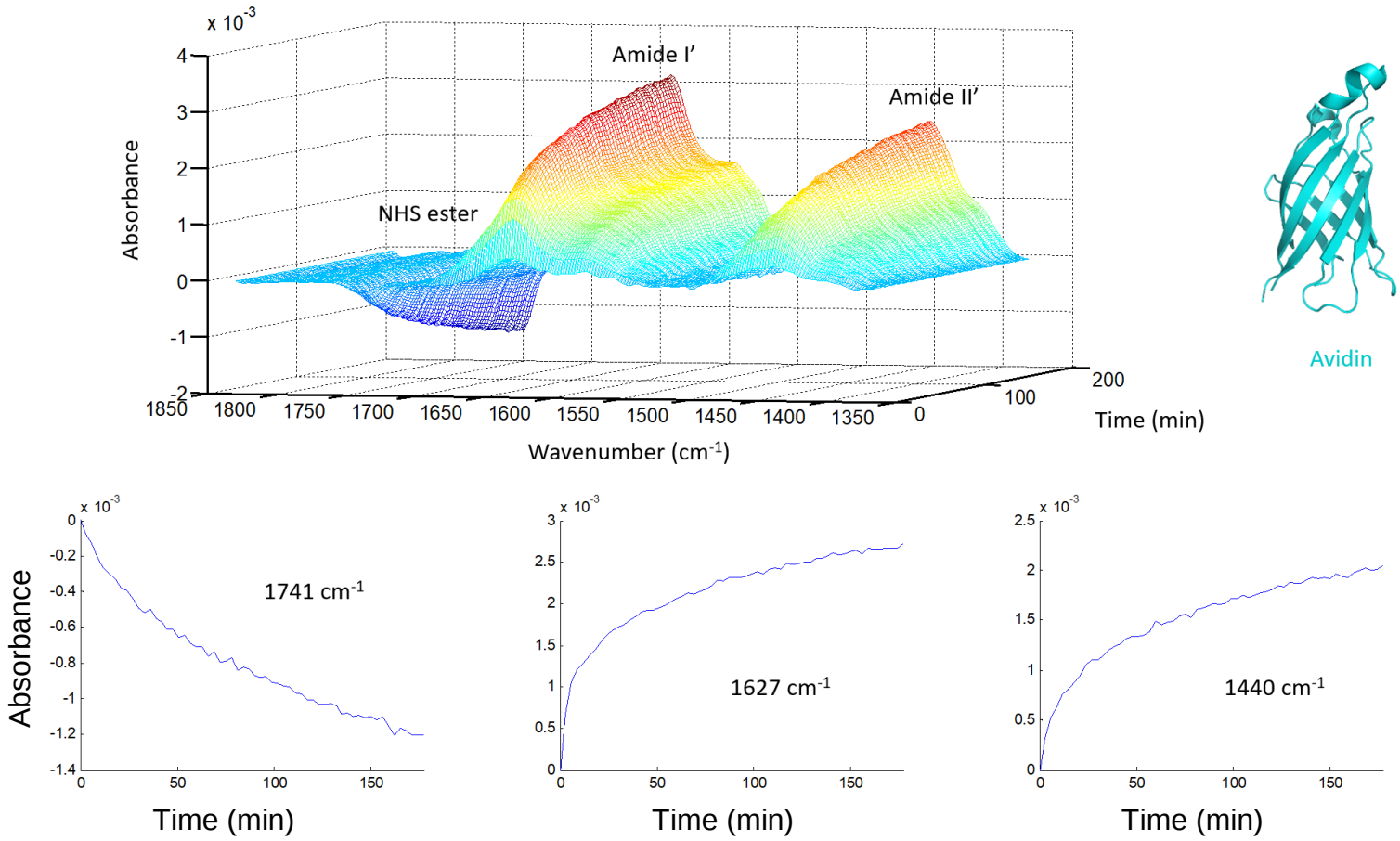


Figure S6: Grafting kinetics of avidin on the activated germanium surface monitored by ATR-FTIR spectroscopy. The color gradient indicates the absorbance intensity. As the NHS ester band at 1741 $cm^{-1}$ disappear in blue, amide bands at 1627 $cm^{-1}$ and 1440 $cm^{-1}$ appear in red over time. Duration of the kinetics is 3 hours, and a spectrum is recorded every 3 min (60 spectra in total). Evolution of the absorbance of the three specific wavenumbers over time (min) is shown below.

**Figure S7: avidin immobilization kinetic correlation**

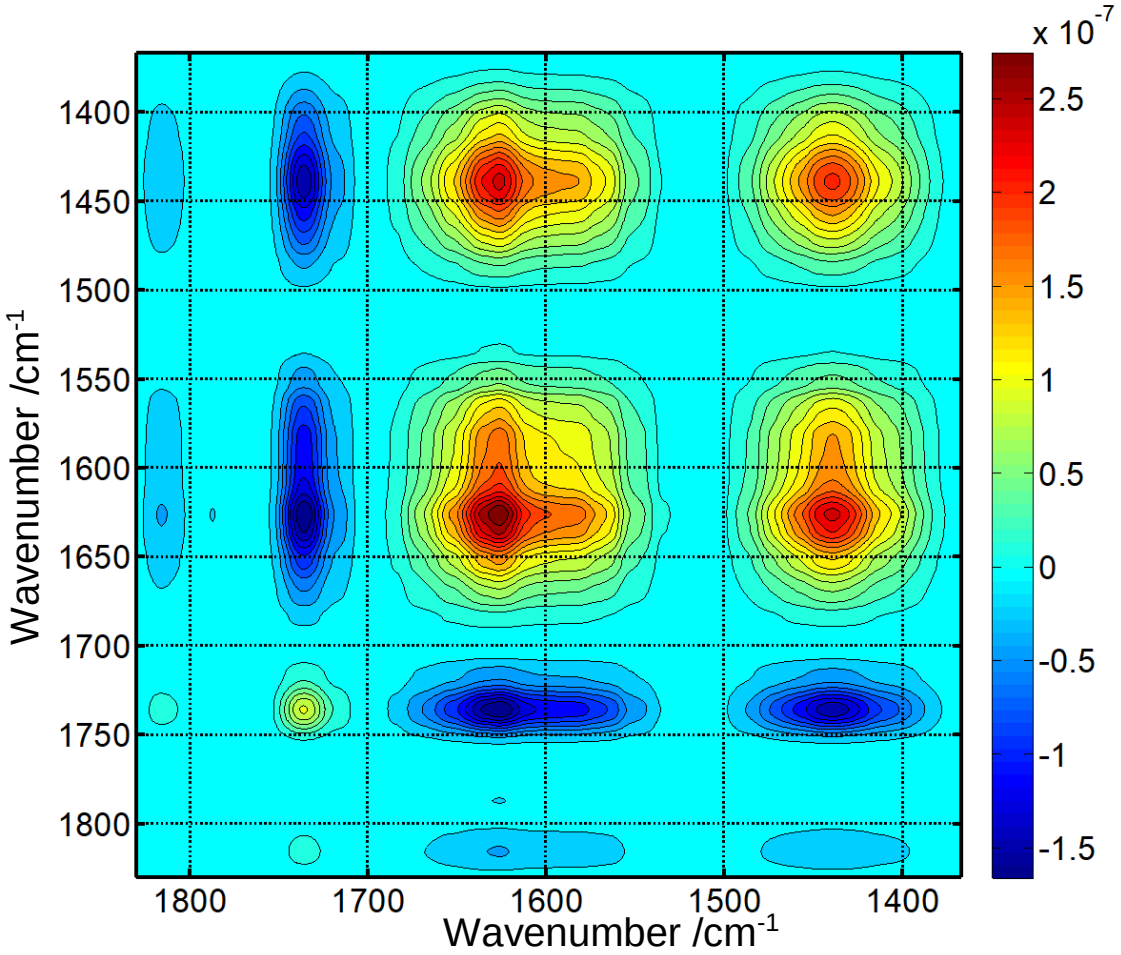


Figure S7: Magnitude of the covariance between the different wavenumbers during the grafting of avidin. The disappearance of the NHS ester at 1741 $cm^{-1}$ is significantly and negatively correlated with the appearance of the amide bands at 1627 $cm^{-1}$ and 1640 $cm^{-1}$. Correlation coefficient between 1741 $cm^{-1}$ and 1627 $cm^{-1}$: -0.967, corresponding p-value: 3.2 $10^{-36}$. Correlation coefficient between 1741 $cm^{-1}$ and 1440 $cm^{-1}$: -0.986, corresponding p-value: 1.310$^{-46}$. Correlation coefficient between 1627 $cm^{-1}$ and 1440 $cm^{-1}$: +0.994, corresponding p-value: 6.310$^{-54}$

**Figure S8: Protein G immobilization**

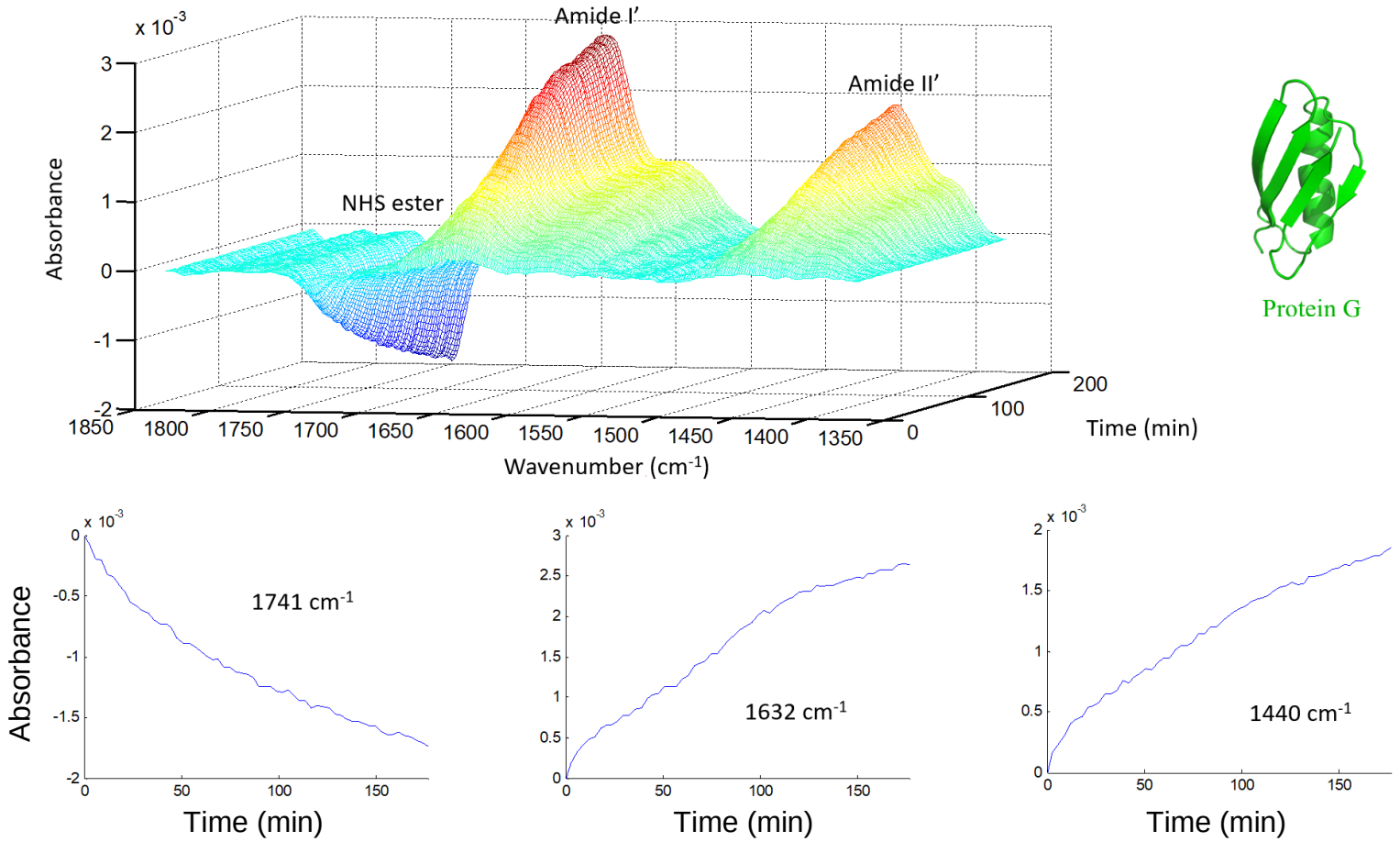


Figure S8: Grafting kinetics of protein G on the activated germanium surface monitored by ATR-FTIR spectroscopy. The color gradient indicates the absorbance intensity. As the NHS ester band at 1741 $cm^{-1}$ disappear in blue, amide bands at 1632 $cm^{-1}$ and 1440 $cm^{-1}$ appear in red over time. Duration of the kinetics is 3 hours, and a spectrum is recorded every 3 min (60 spectra in total). Evolution of the absorbance of the three specific wavenumbers over time (min) is shown below.

**Figure S9: Preservation of the structural integrity**

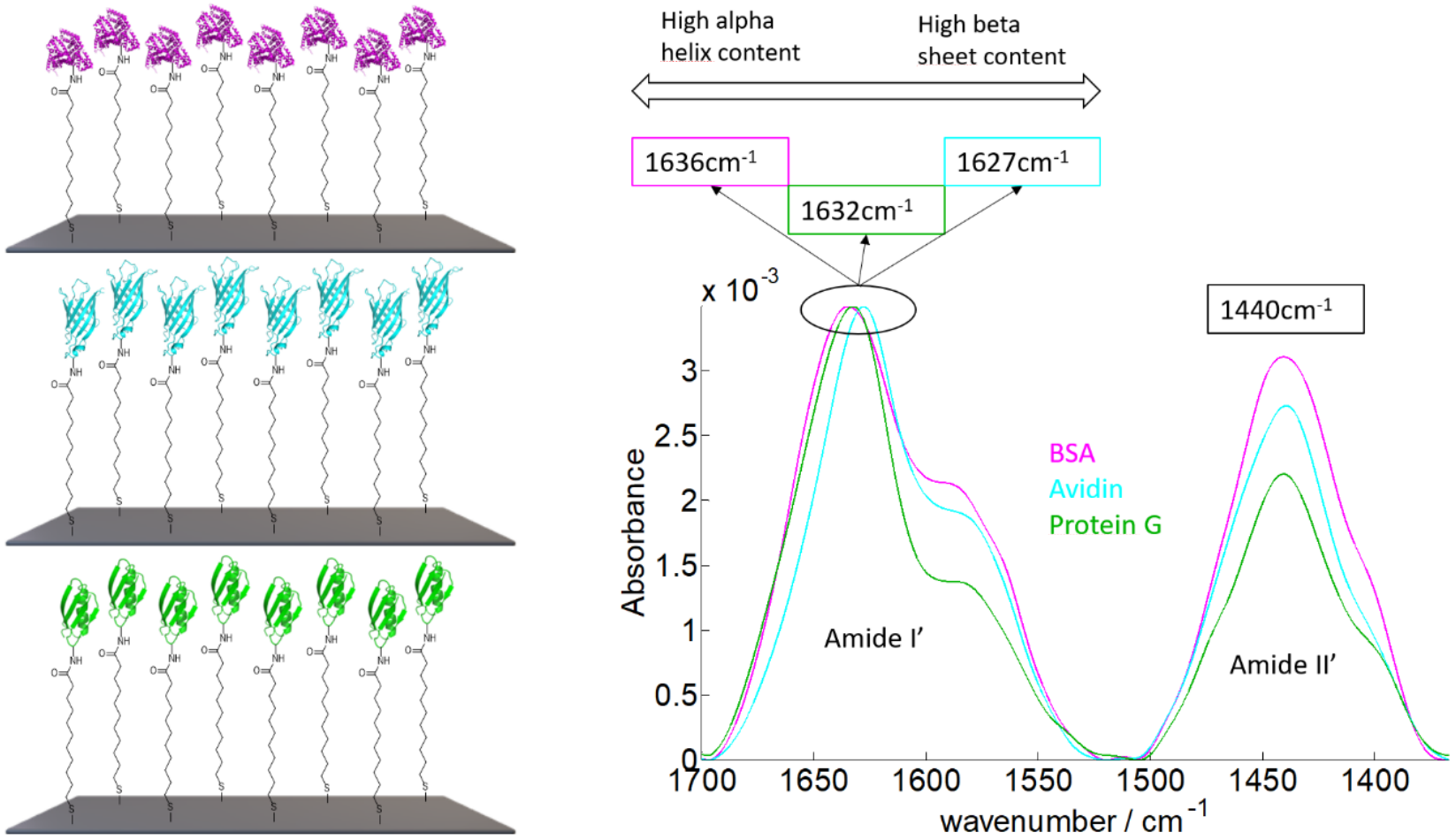


Figure S9: FTIR spectra of the immobilization of three very different proteins in terms of secondary structure. Bovin Serum Albumin (purple) is mainly composed of alpha-helices, avidin (blue) contains mainly beta-sheets and the immunoglobulin-binding protein, protein G (green) contains a mix of both. The wavelength of the Amide I' band of each protein is consistent with its secondary structure content.

**Figure S10: mAb grafting control**

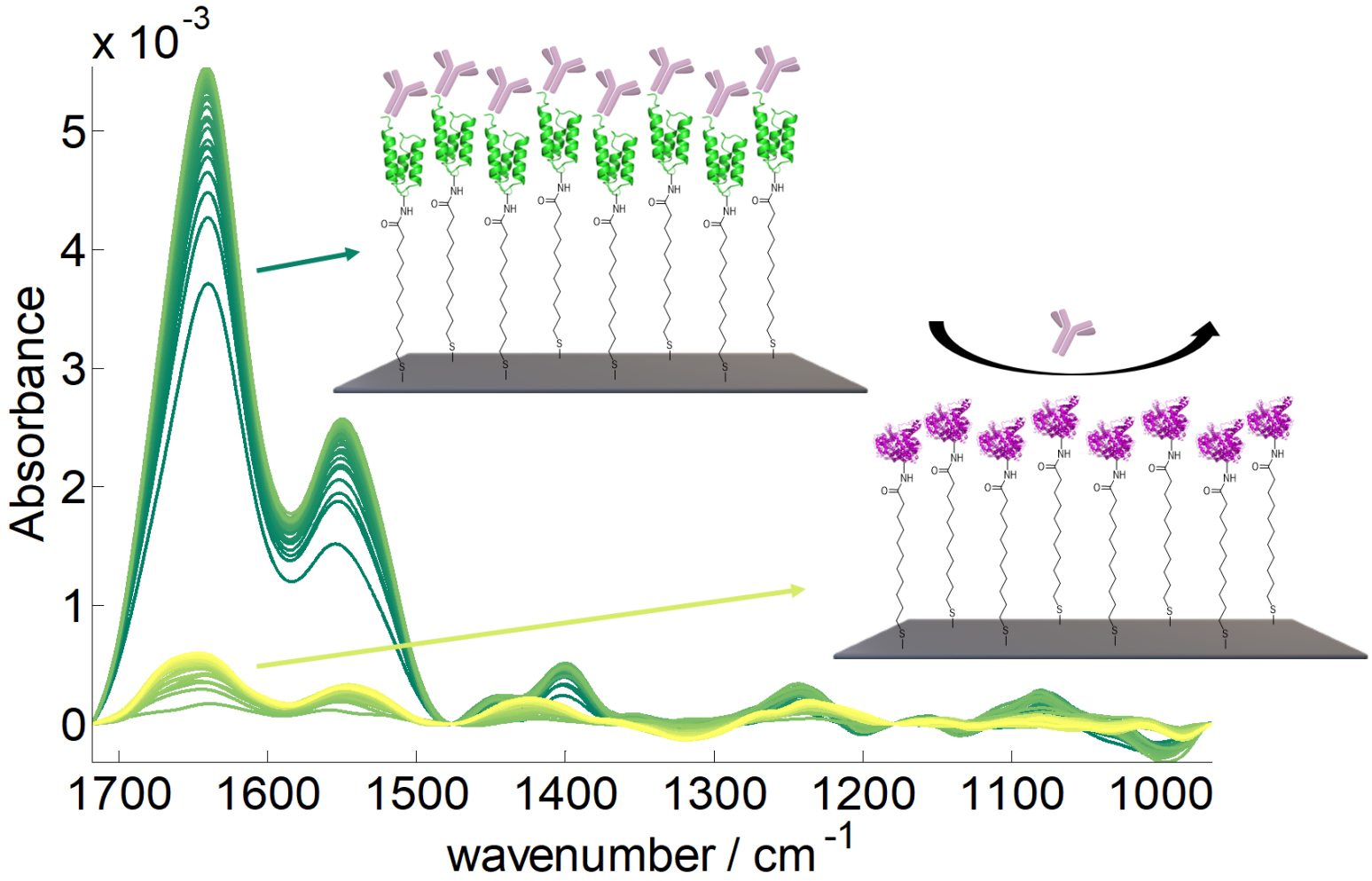


Figure S10: Specificity of antibody binding from a complex culture medium onto the developed biosensor. The series of spectra (in green) depicts the binding of antibodies present in a culture medium onto a protein A coated germanium surface as a function of the time. On a BSA coated surface (series of spectra in yellow) no significant binding of antibodies occurred.

**Figure S11: mAb grafting control**

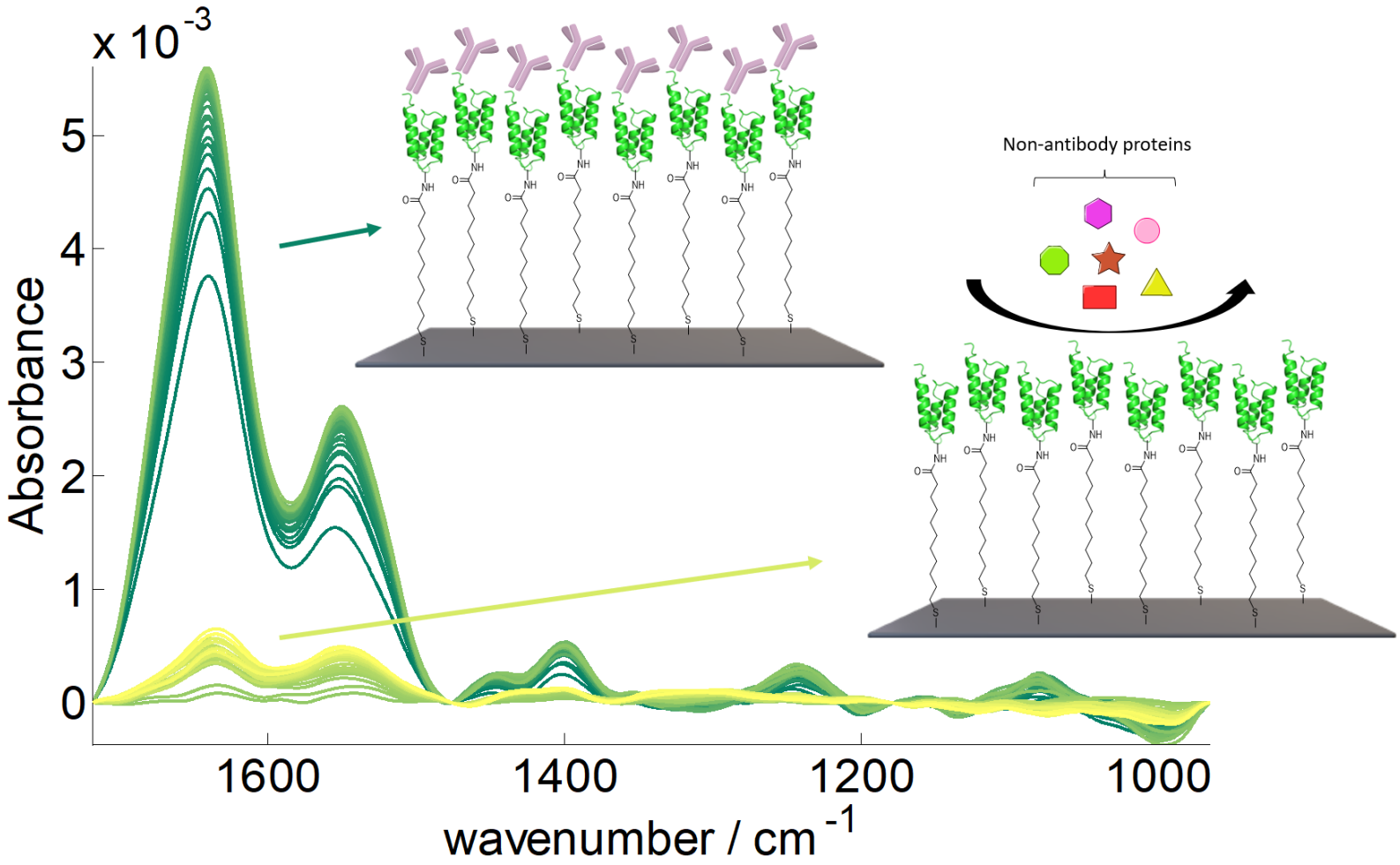


Figure S11: Specificity of antibody binding from a complex culture medium onto the developed biosensor. The series of spectra in green reports the binding of antibodies from a culture medium at day 16 of growth onto the protein A coated germanium surface. Spectra are the same as those reported in Figure S10. When a culture medium at day 0 containing no antibody (series of spectra in yellow) was used, no significant binding of antibodies occurred.