**Supplementary Materials**

# Enhanced enzyme reuse through the bioconjugation of L-asparaginase and silica-based supported ionic-liquid like phase materials

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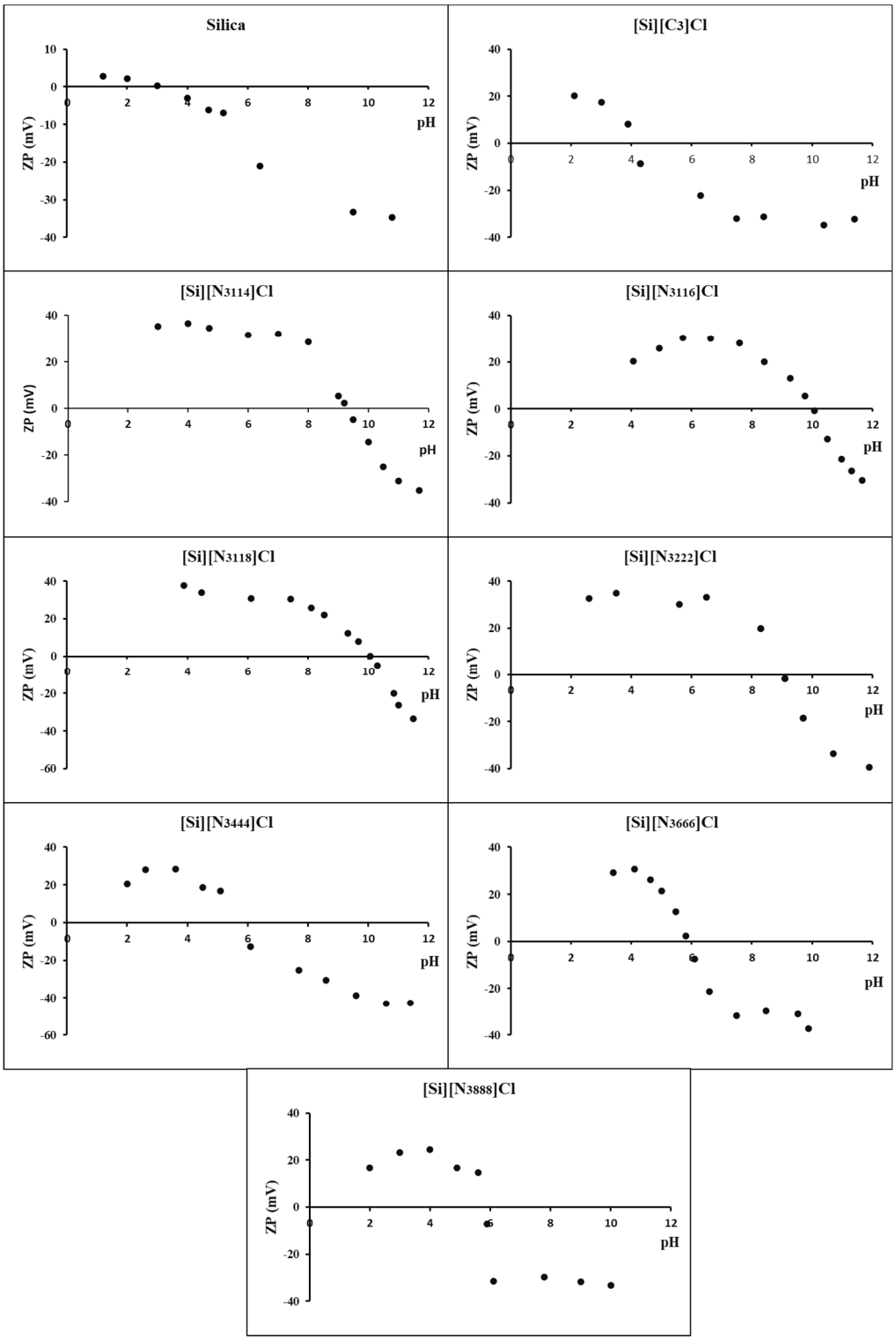


**Figure S1.** Zeta potential (ZP) of silica, [Si][$C_3$]Cl and all SSILLP materials ([Si][$N_{3114}$]Cl, [Si][$N_{3116}$]Cl, [Si][$N_{3118}$]Cl, [Si][$N_{3222}$]Cl, [Si][$N_{3444}$]Cl, [Si][$N_{3666}$]Cl and [Si][$N_{3888}$]Cl) as a function of pH.

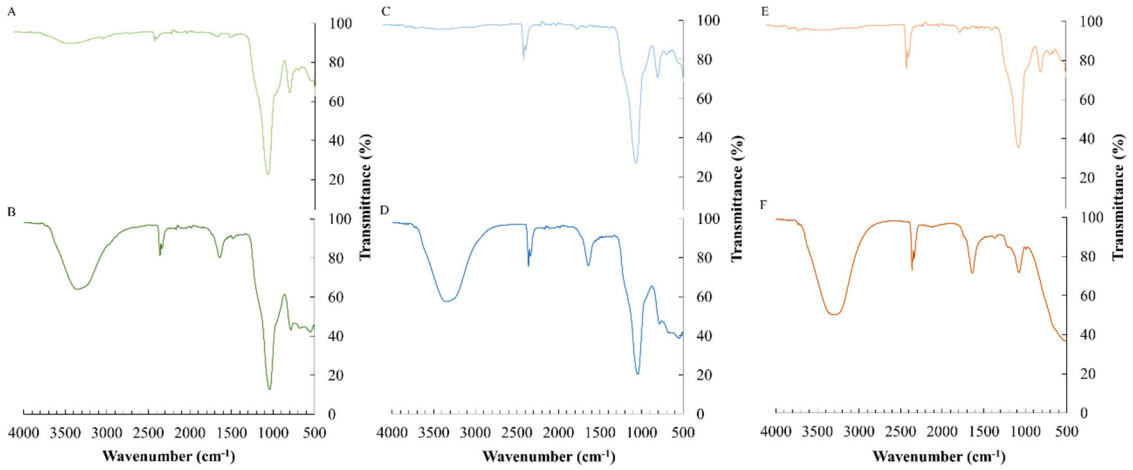


**Figure S2.** Attenuated total reflectance - Fourier-transform infrared (ATR-FTIR) spectra of [Si][$N_{3114}$]Cl (A), ASNase-[Si][$N_{3114}$]Cl (B), [Si][$N_{3222}$]Cl (C), ASNase-[Si][$N_{3222}$]Cl (D), [Si][$N_{3888}$]Cl (E), and ASNase-[Si][$N_{3888}$]Cl (F).

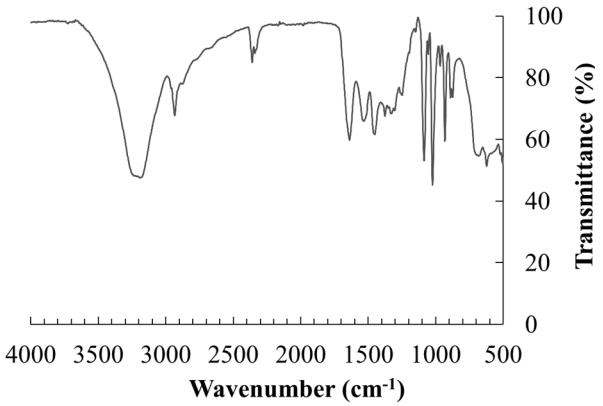


**Figure S3.** ATR-FTIR spectrum of the commercial ASNase from *E. coli* used in this work.

The relative recovered activity of immobilized ASNase ($RRA_{IA}$) (%) onto the SSILLP materials was assessed using the following conditions: constant ASNase concentration ($6 \times 10^{-2}$ mg $mL^{-1}$), volume of the Nessler reaction ($V_N$: 2.5 mL), volume of the stopped reaction sample ($V_S$: 0.1 mL), reaction time ($R_T$: 30 min) and was determined according to Eq. S1:

$$RRA_{IA} = \frac{A_{IA}}{MA_{FA}} \times 100 \quad (S1)$$

where $A_{IA}$ (U $mg^{-1}$) is the activity of the effectively immobilized enzyme and $MA_{FA}$ (U $mg^{-1}$) is the maximum theoretical activity that would exist if the free enzyme was totally immobilized (U $mg^{-1}$) determined according to Eqs. S2 and S3:

$$A_{IA} = \frac{NH_4^+ \times V_R}{M_M} \quad (S2)$$

$$MA_{FA} = \frac{NH_4^+ \times d_f}{M_M} \quad (S3)$$

where $NH_4^+$ is the ammonium concentration produced in the enzymatic reaction (μmol $mL^{-1}$), $V_R$ is the volume of enzymatic reaction (1 mL), $M_M$ is the mass of the SSILLP materials (10 mg) and $d_f$ is the dilution factor.

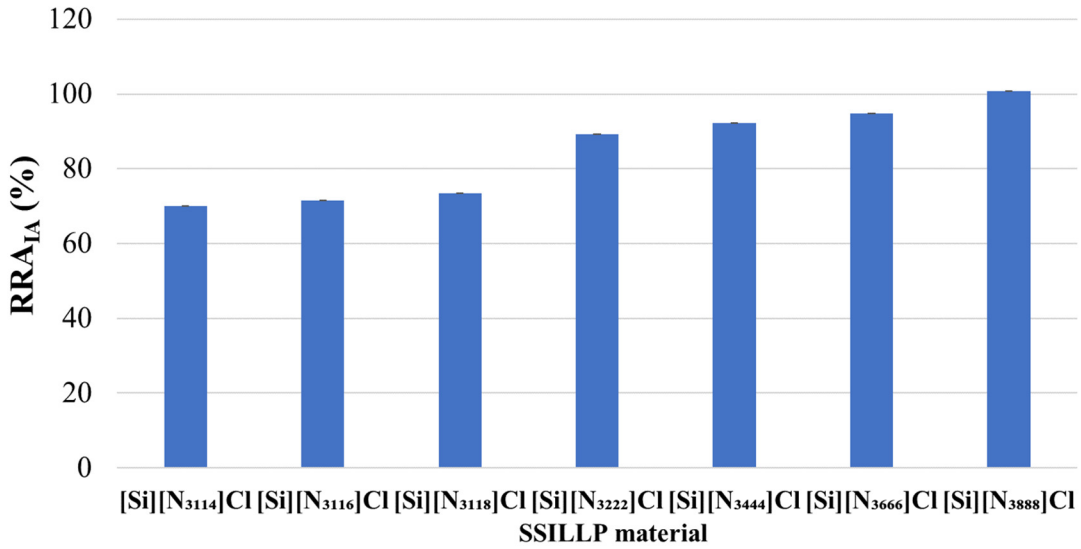


**Figure S4.** Relative recovered activity of immobilized ASNase ($RRA_{IA}$) on the supports: [Si][$N_{3114}$]Cl, [Si][$N_{3116}$]Cl, [Si][$N_{3118}$]Cl, [Si][$N_{3222}$]Cl, [Si][$N_{3444}$]Cl, [Si][$N_{3666}$]Cl and [Si][$N_{3888}$]Cl under optimized assay conditions (pH 8, $6 \times 10^{-3}$ mg $mg^{-1}$ of ASNase and 60 min). Error bars correspond to standard deviation.

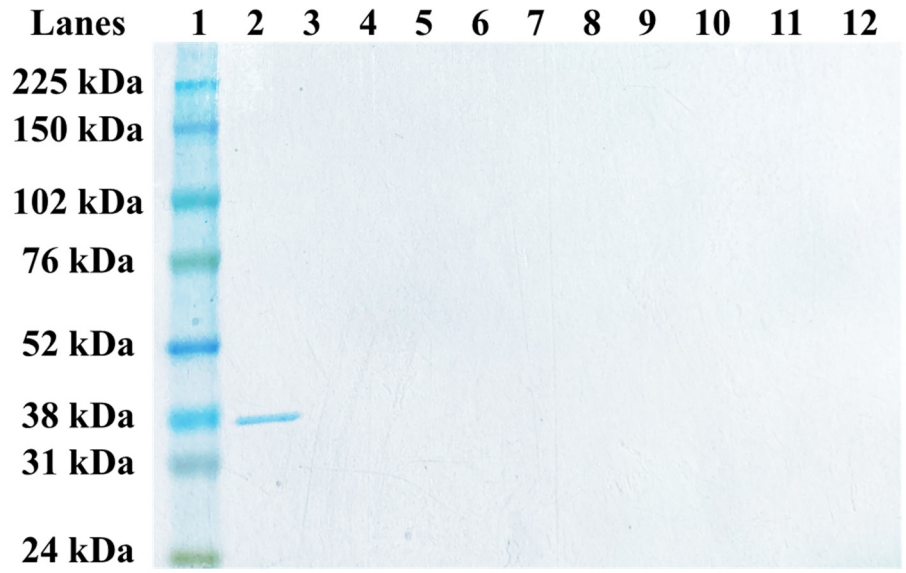


**Figure S5.** SDS-Page of the supernatants of the assays performed in the operational stability studies (5 cycles of reaction) for the SSILLP materials [Si][$N_{3888}$]Cl and [Si][$N_{3114}$]Cl; lane 1: Full-Range Rainbow Molecular Weight Marker by Cytiva (protein molecular weight marker), lane 2: 0.06 mg $mL^{-1}$ of ASNase, lanes 3, 4, 5, 6, and 7: cycles 1, 2, 3, 4, and 5 of [Si][$N_{3888}$]Cl, lanes 8, 9, 10, 11, 12: cycles 1, 2, 3, 4, and 5 of [Si][$N_{3114}$]Cl.