Electronic Supplementary Material (ESI) for CrystEngComm.

Enhancing the crystallization of insulin using amino acids as soft-templates to control nucleation

Frederik J. Link[a] and Jerry Y. Y. Heng[a,b*]

[a]Department of Chemical Engineering and [b]Institute for Molecular Science and Engineering, Imperial College London, London SW7 2AZ, UK.

Supporting Information

S 1. Crystal occurrence of human insulin as a dependence of arginine, glycine and leucine concentration after 4 and 7 days

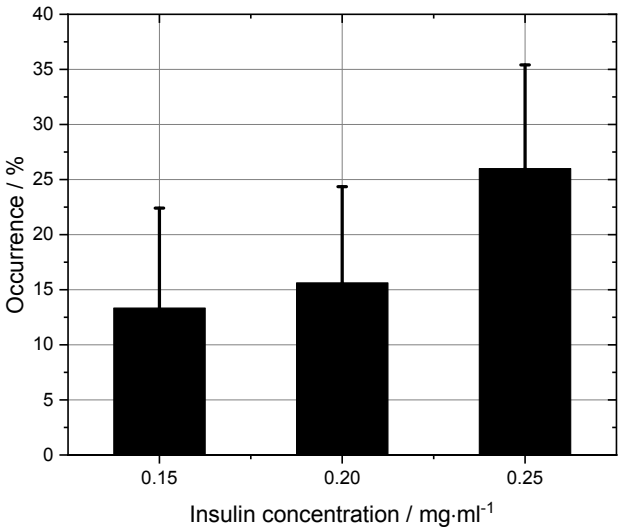

Figure 1: Occurrence and standard deviation of insulin crystals after 4 days at different initial insulin concentrations without amino acids.

Table 1: Crystal occurrence and standard deviation of occurrence of human insulin as a dependence of arginine, glycine and leucine concentration after 4 days

Amino acid type	0	0.01 M	0.03 M	0.06 M	0.1 M
-	15.6 ± 8.7%				
Arginine		100.0 ± 0 %	100.0 ± 0 %	100.0 ± 0 %	81.0 ± 17.1 %
Glycine		24.7 ± 23.7 %	9.3 ± 8.8 %	14.1 ± 9.5 %	32.3 ± 27.3 %
Leucine		21.9 ± 14.7 %	32.1 ± 24.6 %	59.8 ± 24.2 %	72.7 ± 20.3 %

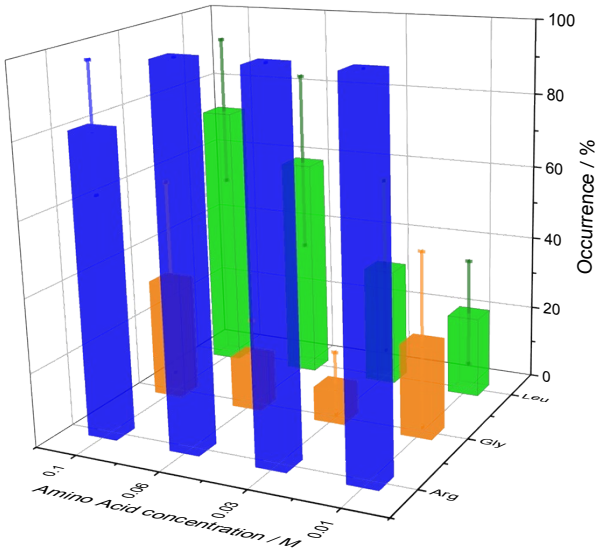

Figure 2: Crystal occurrence and standard deviation of occurrence of human insulin as a dependence of arginine, glycine and leucine concentration after 4 days

Table 2: Crystal occurrence and standard deviation of human insulin as a dependence of arginine, glycine and leucine concentration after 7 days.

Amino acid type	0	0.01 M	0.03 M	0.06 M	0.1 M
-	15.6 ± 8.7%				
Arginine		100.0 ± 0 %	100.0 ± 0 %	100.0 ± 0 %	85.7 ± 10.4 %
Glycine		26.0 ± 22.9 %	11.1 ± 9.5 %	14.1 ± 9.5 %	33.9 ± 30.1 %
Leucine		21.9 ± 14.7 %	34.5 ± 23.5 %	61.6 ± 26.2 %	72.7 ± 20.3 %

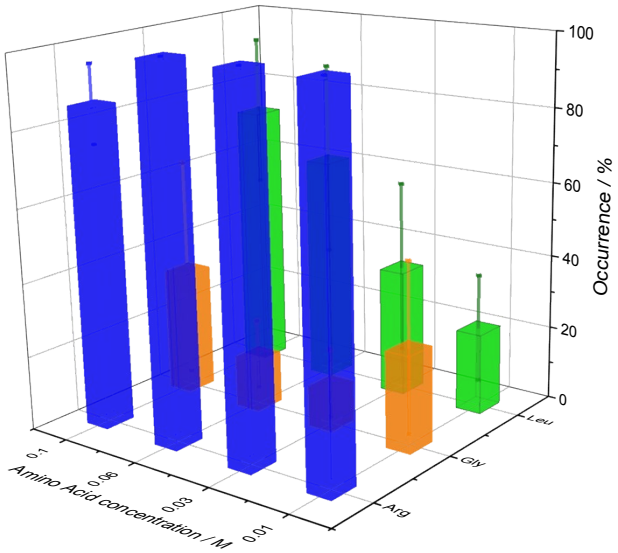

Figure 3: Crystal occurrence and standard deviation of human insulin as a dependence of arginine, glycine and leucine concentration after 7 days

Table 3: Crystal occurrence and standard deviation of human insulin in addition of 0.3 M arginine after 4 and 7 days

4 days	0 ± 0 %
7 days	4.2 ± 4.2 %

S 2. HPLC analysis for insulin concentration measurement

Chromatographic conditions utilised

- Column: Phenomenex Luna C-18 (average particle size 5 µm, pore size 100 A) column (150 x 4.6 mm)
- Mobile Phase: 62% v/v KH_2PO_4 (0.1 M), 26 % v/v acetonitrile, 12 % v/v Methanol
- Flowrate: 0.75 ml/min
- Wavelength: 214 nm
- Column temperature: 35 degree Celsius
- Sample injection volume: 20 µL

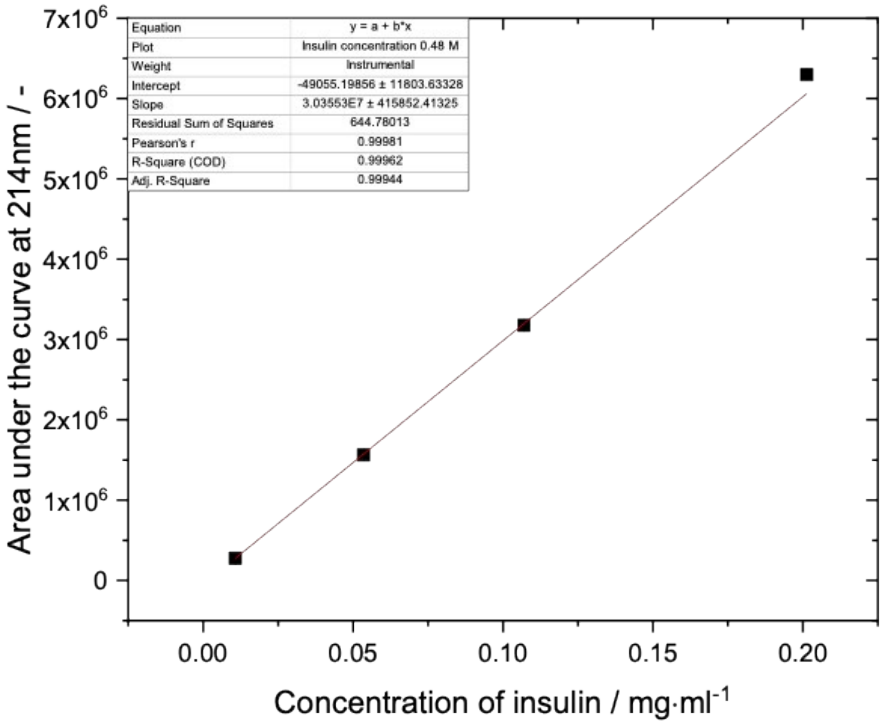

Figure 4: Linear standard curve for insulin at retention time of 12.7 minutes.

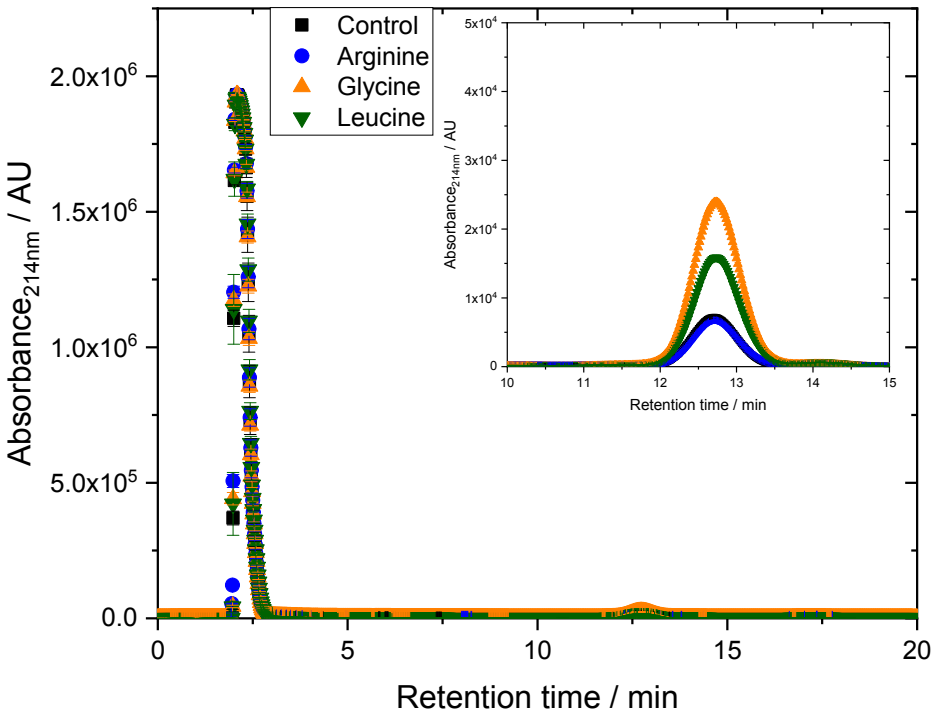

Figure 5: Absorbance spectrum for pure human insulin (control) and human insulin in addition of 0.03 M of arginine, glycine and leucine.