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Affinity Peptide Extraction Using Small-Molecule, Thermoresponsive Zwitterionic Liquid Materials

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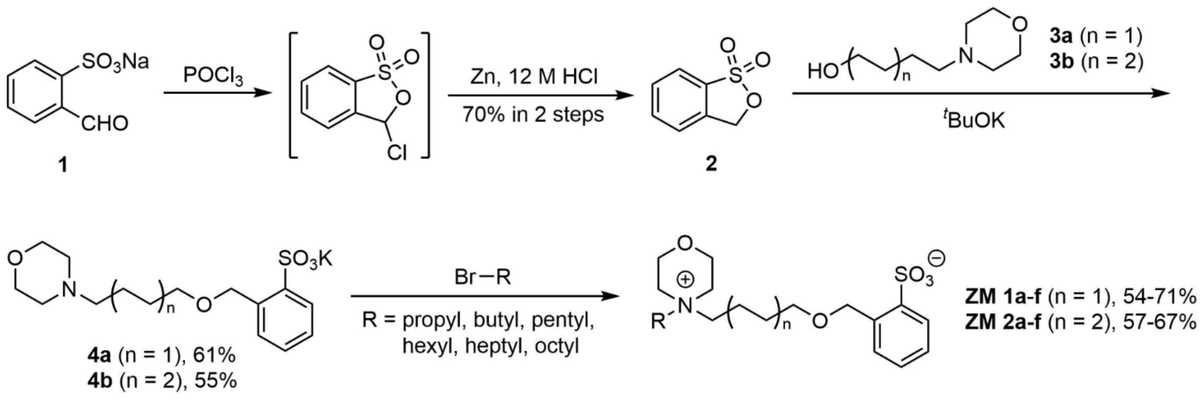
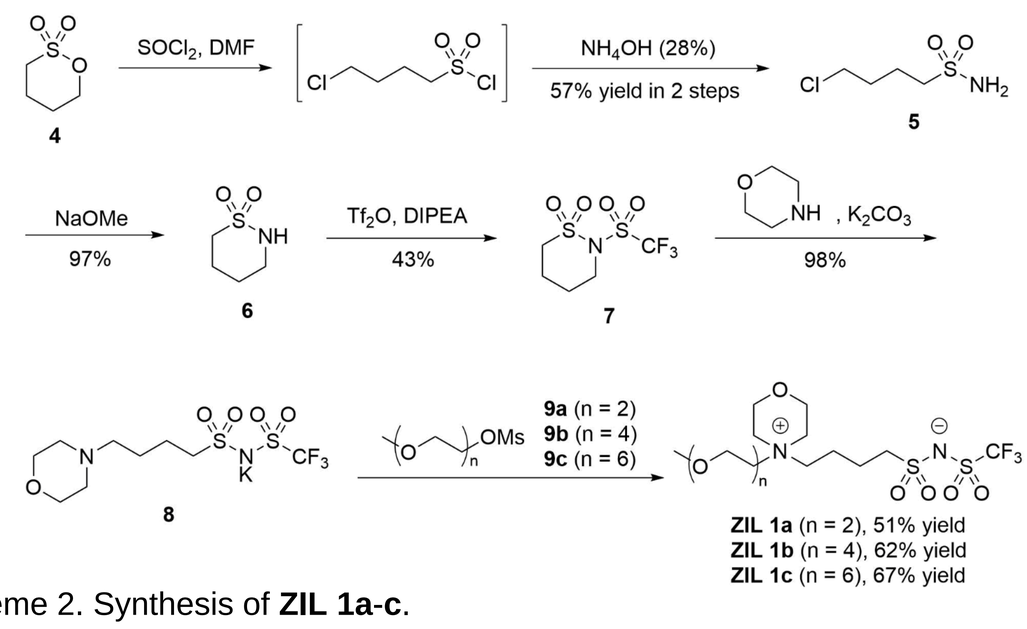
The zwitterionic liquid materials are small-molecule newfangled organic salts and can be used as biocompatible solvents for biomolecule extraction. This poster presents the use of thermoresponsive ionic materials with LCST (lower critical solution temperature) properties for water-ionic liquid extraction aiming at both biomolecule enrichment and affinity extraction. Additionally, in affinity extraction, a small amount of **CIL1** (crowned ionic liquid) is added to utilize its crown ether cavity and cation-chelating ability for extracting biomolecules from aqueous solutions.

Synthesis of Zwitterionic Liquid Materials (ZMs)



Scheme 1. Synthesis of **ZM 1a-f** and **ZM 2a-f**.



Scheme 2. Synthesis of **ZIL 1a-c**.

Results and Discussion

We successfully synthesized a small library of 12 ZMs, and discovered 3 ZMs (**ZM 1e**, **ZM 1f**, and **ZM 2d**) carrying LCST phase separation in water. A QR code allows to readily view the video actions of LCST phase transition with heating and cooling of **ZM 1f** in water.

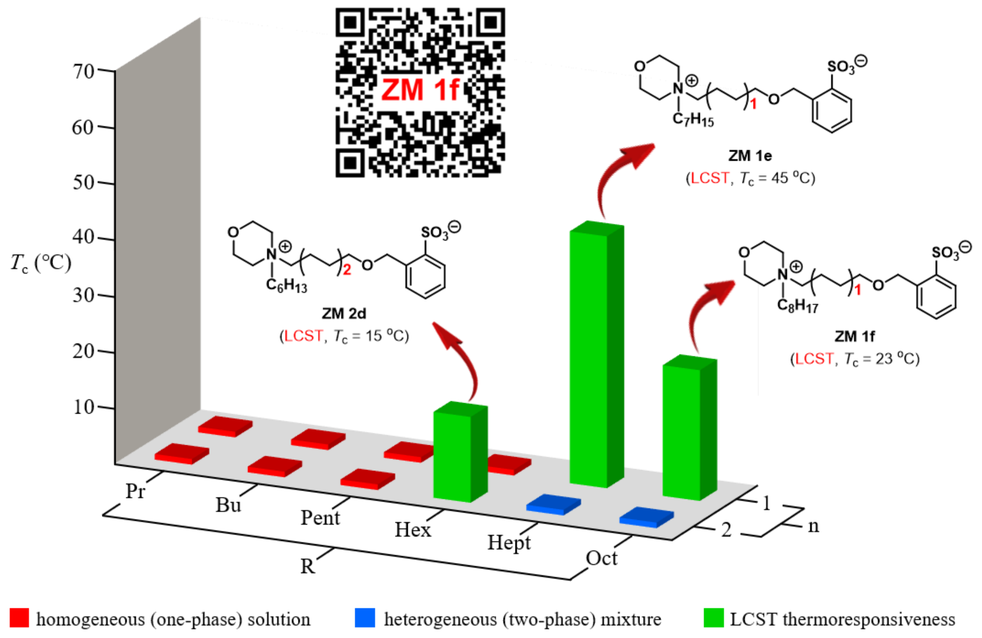


Figure 1. Thermoresponsive materials screening of a small library of 12 zwitterionic morpholinium sulfonates (**ZM 1a-f** and **ZM 2a-f**) upon mixing with water at temperatures between 4 ℃ and 90 ℃. With this library, three ZMs (labeled in green) show LCST phase transitions: **ZM 1e** (T_c = 45 ℃), **ZM 1f** (T_c = 23 ℃) and **ZM 2d** (T_c = 15 ℃).

Concentration enrichment of cytochrome c by ZM 2d

Figure 2 shows that **ZM 2d** is miscible with an aqueous cytochrome c solution (1:10, w/w) at low temperature (4 ℃) but undergoes LCST phase separation upon heating to 37 ℃. The result suggested that a highly diluted protein solution was readily enriched 16-fold. Furthermore, after the enrichment, cytochrome c maintains its original structure (no change of the Soret absorption) and is not destroyed.

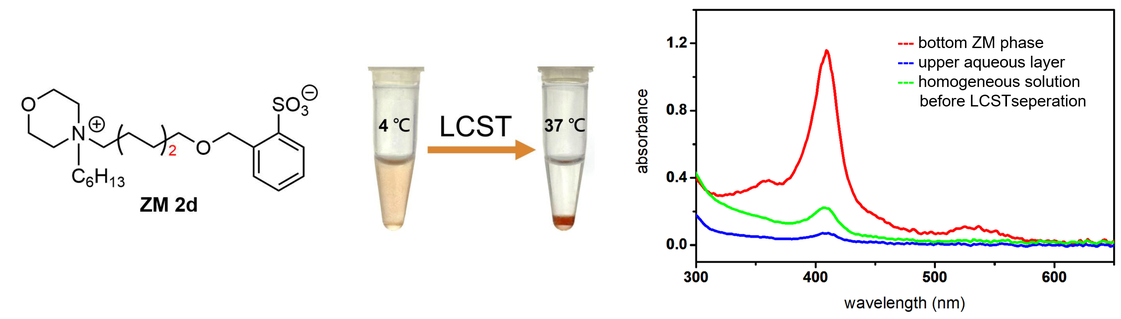


Figure 2. Photos and UV-vis spectra of **ZM 2d** enriched cytochrome c during its transition from the aqueous phase to ionic liquid phase via LCST mixing and separation.

Affinity extraction of peptides and protein by CIL 1

Figure 4 shows the extraction of peptides (Dab-$(R)_6$) and proteins (cytochrome c) using **ZIL 1b** (immiscible with water) and **ZIL 1c** (with LCST properties, T_c = 26 ℃). First, the ZILs are mixed with the aqueous solution containing biomolecules for extraction, where the biomolecules remain in the aqueous phase. Next, the affinity ionic liquid **CIL 1** is added for a chemoselective extraction based on biomolecular recognition, eventually resulting in the transfer of the biomolecules into the ionic liquid layer. Finally, by adding KCl (for peptides) or NH_4CO_2 (for cytochrome c), the biomolecules can be back-extracted into the aqueous phase while retaining their original structures.

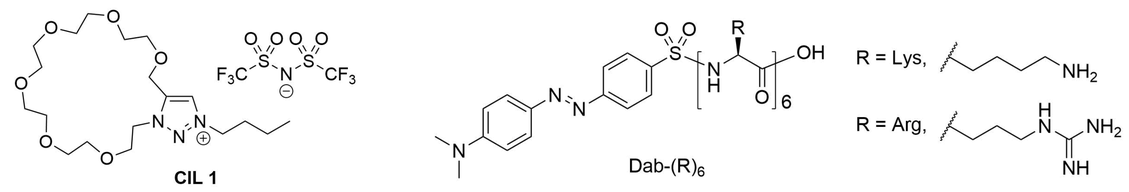


Figure 3. The structure of **CIL 1** and Dab-$(R)_6$

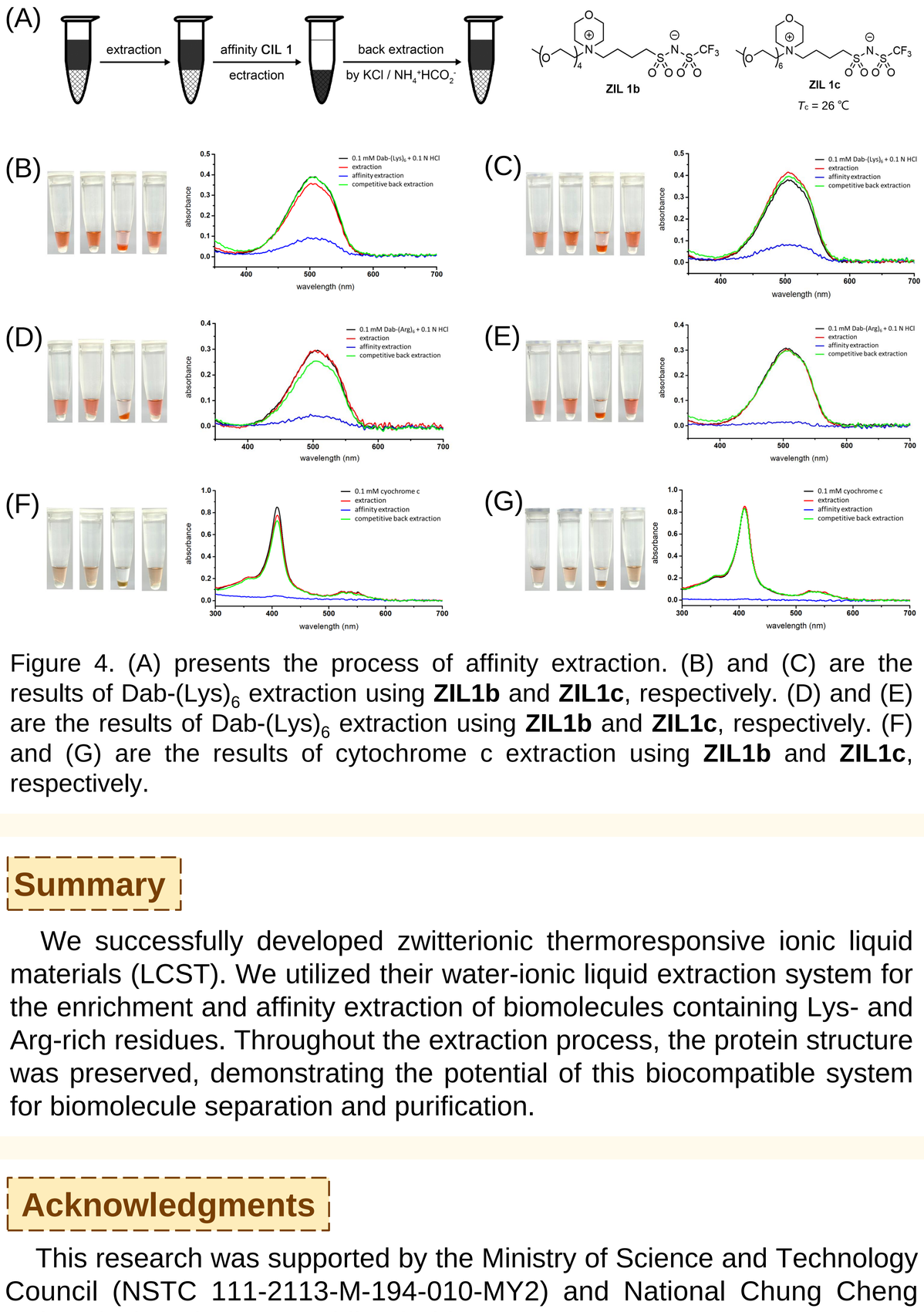


Figure 4. (A) presents the process of affinity extraction. (B) and (C) are the results of Dab-$(Lys)_6$ extraction using **ZIL1b** and **ZIL1c**, respectively. (D) and (E) are the results of Dab-$(Lys)_6$ extraction using **ZIL1b** and **ZIL1c**, respectively. (F) and (G) are the results of cytochrome c extraction using **ZIL1b** and **ZIL1c**, respectively.

Summary

We successfully developed zwitterionic thermoresponsive ionic liquid materials (LCST). We utilized their water-ionic liquid extraction system for the enrichment and affinity extraction of biomolecules containing Lys- and Arg-rich residues. Throughout the extraction process, the protein structure was preserved, demonstrating the potential of this biocompatible system for biomolecule separation and purification.

Acknowledgments

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NSTC 國家科學及技術委員會 National Science and Technology Council

國立中正大學 National Chung Cheng University