# Thermo-responsive, self-assembling biointerface for on demand release of surface-immobilised proteins

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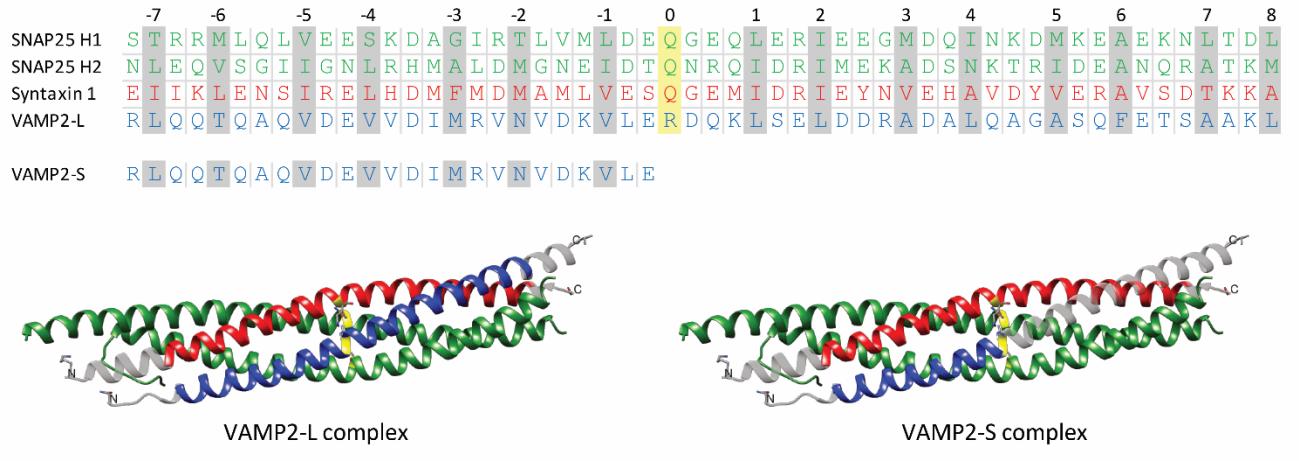
## Supplementary Tables

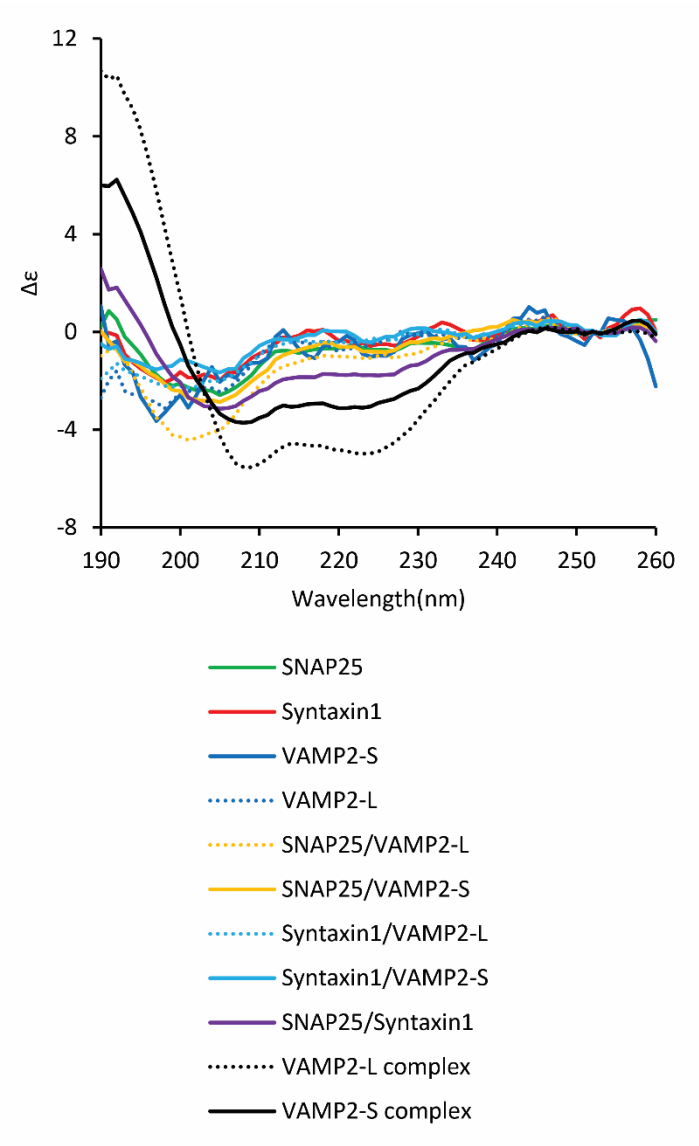
| Protein | Amino acid sequence | MW (kDa) |
|---|---|---|
| SNAP25 | GSMAEDADMRNELEEMQRRADQLADESLESTRRMLQLVEESKDAGIRTLVMLDEQGEQLERIEEGMDQINKDMKEAEKNLTDLGKFAGLAVAPANKLKSSDAYKKAWGNNQDGVVASQPARVVDEREQMAISGGFIRRVTNDARENEMDENLEQVSGIIGNLRHMALDMGNEIDTQNRQIDRIMEKADSNKTRIDEANQRATKMLGSG | 23.3 |
| VAMP2-S | GSRLQQTQAQVDEVVDIMRVNVDKVLECGS | 3.3 |
| VAMP2-L | GSRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDDRADALQAGASQFETSAAKLCGS | 6.4 |
| Syntaxin 1 | GSEIIKLENSIRELHDMFMDMAMLVESQGEMIDRIEYNVEHAVDYVERAVSDTKKAGS | 6.6 |
| Syntaxin 3 | GSDIVRLESSIKELHDMFMDIAMLVENQGEMLDNIELNVMHTVDHVEKARDETKRAGS | 6.6 |
| VS-S | GSRLQQTQAQVDEVVDIMRVNVDKVLECGSGKFAGLAVAPANKLKSSDAYKKAWGNNQDGVVASQPARVVDEREQMAISGGFIRRVTNDARENEMDEGSDIVRLESSIKELHDMFMDIAMLVENQGEMLDNIELNVMHTVDHVEKARDETKRAGS | 17.2 |
| VS-L | GSRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDDRADALQAGASQFETSAAKLCGSGKFAGLAVAPANKLKSSDAYKKAWGNNQDGVVASQPARVVDEREQMAISGGFIRRVTNDARENEMDEGSDIVRLESSIKELHDMFMDIAMLVENQGEMLDNIELNVMHTVDHVEKARDETKRAGS | 21.3 |
| VS-L2 | GSRLQQTQAQVDEVVDIMRVNVDKVLERDQKLSELDDRADALQAGASQFETSAAKLCGSGGGGSGGGSGGGGSGGAGSGGGAGGSGGGSAGGGGSGGAGSGGGSGGGSDIVRLESSIKELHDMFMDIAMLVENQGEMLDNIELNVMHTVDHVEKARDETKRAGS | 16.1 |
| GST | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPGISGGGGGILDSMGRLELKLNSS | 28.6 |
| HH | GSSTRRMLQLVEESKDAGIRTLVMLDEQGEQLERIEEGMDQINKDMKEAEKNLTDLGSGKFAGLAVAPANKLKSSDAYKKAWGNNQDGVVASQPARVVDEREQMAISGGFIRRVTNDARENEMDEGSNLEQVSGIIGNLRHMALDMGNEIDTQNRQIDRIMEKADSNKTRIDEANQRATKMGS | 20.3 |
| GST-HH | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSSTRRMLQLVEESKDAGIRTLVMLDEQGEQLERIEEGMDQINKDMKEAEKNLTDLGSGKFAGLAVAPANKLKSSDAYKKAWGNNQDGVVASQPARVVDEREQMAISGGFIRRVTNDARENEMDEGSNLEQVSGIIGNLRHMALDMGNEIDTQNRQIDRIMEKADSNKTRIDEANQRATKMGS | 45.3 |

**Supplementary Table 1**. List of all the proteins used, their amino acid sequence and their expected molecular weight calculated using the translated nucleotide sequence and ProtParam (https://web.expasy.org/protparam/). Colour code: grey = unstructured linkers, dark green = N-terminal H domain of SNAP25, light green = C-terminal H domain of SNAP25, yellow A = cysteine to alanine substitutions, yellow C = cysteine residues for site specific labelling, blue = VAMP2 SNARE domains, red = syntaxin SNARE domains, orange = GST. To produce the VS fusion proteins, Syntaxin 3 was used in place of Syntaxin 1, as it was previously reported that Syntaxin 1 fusions have lower expression in bacteria (Darios, F. et al. SNARE Tagging Allows Stepwise Assembly of a Multimodular Medicinal Toxin. *Proc. Natl. Acad. Sci.* **2010**, *107*, 18197–18201). To confirm that Syntaxin 3 assembles with SNAP25 and VAMP2, SRCD was performed and the far-UV spectra confirmed that there is no difference between Syntaxin 1 and Syntaxin 3 in their ability to form a SNARE complex (Supplementary Figure 3).

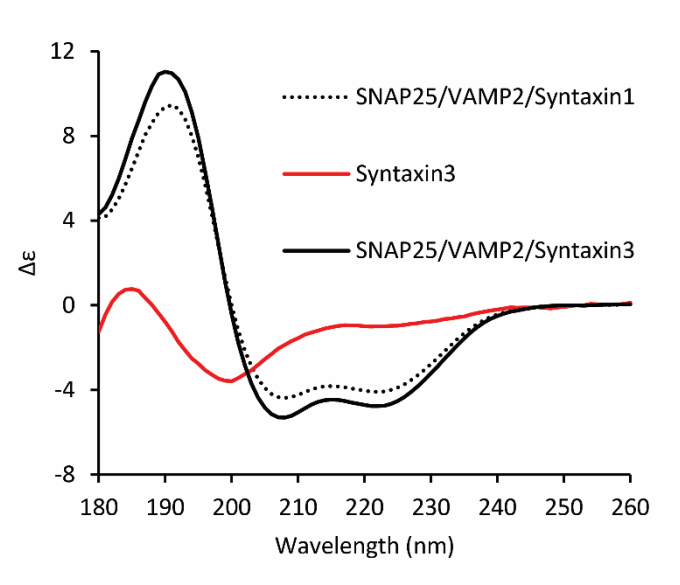
**Supplementary Figures**



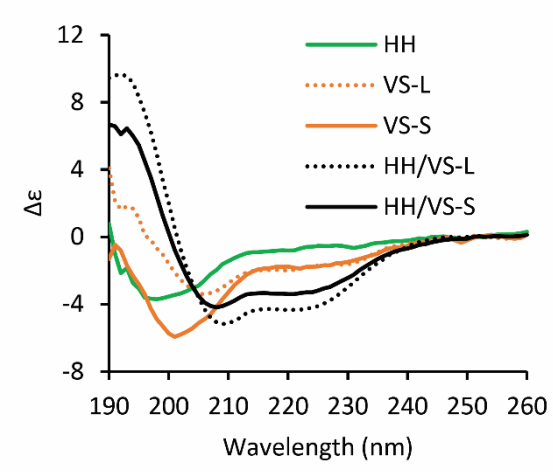

**Supplementary Figure 1**. Truncation of the native SNARE complex. The figure shows the alignment (N-terminal to C-terminal) of the two SNARE domains of SNAP25 (H1 and H2, green), the SNARE domain of Syntaxin 1 (red) and VAMP2 (blue). The long (VAMP2-L) and short (VAMP2-S) versions of the latter are compared to illustrate the extent of the truncation, which was aimed at making a SNARE complex with lower thermal stability (VAMP2-S complex). The sequences are highlighted and colour coded on the complex structures (PDB ID: 1SFC) underneath the alignment schematic. The SNARE complex has parallel alignment and, in this representation, the N-terminals of each polypeptide are all on the left. The N- and C-terminals of the SNARE domain polypeptides were chosen to span from the first coil (N-terminal) of the VAMP2 to the last coil (C-terminal) of Syntaxin 1, while preserving the same overall polypeptide length. This represents the 'core' SNARE complex, which presents typical features of a coiled-coil structure. Coiled-coils are made of aligned motifs with multiple heptad repeats (7 amino acids), where hydrophobic amino acids are over-represented in positions 1 and 3. As a consequence of this pattern, the hydrophobic amino acids on different α-helices face each other within the core of the coiled-coil structure and the α-helices "zip" together via the formation of hydrophobic layers (two per heptad, highlighted in grey in the alignment schematic). The core SNARE complex represented above has 16 layers, one of which is not hydrophobic (highlighted in yellow in the figure), is referred to as "ionic" layer or "zero" layer and is a conserved feature of SNARE domains. The hydrophobic layers are numbered with negative (towards the N-terminal) or positive numbers (towards the C-terminal) depending on their position relative to the "zero" layer. Previous studies show that SNARE complex less stable than the native one can be obtained by systematically reducing the number of complete hydrophobic layers (Ferrari, E. et al. Assembly of Protein Building Blocks Using a Short Synthetic Peptide. *Bioconjug. Chem.* **2012**, *23*, 479–484). SNARE complex with reduced stability were obtained by shortening the C-terminal side of just one SNARE polypeptide. N-terminal truncation would instead prevent the formation of the SNARE complex, as the self-assembling starts at the N-terminal and proceeds towards the C-terminal. The same study also determined that a truncation that includes only the layers -7 to -1 self-assembles more effectively and yields better binding properties compared to truncated SNARE complexes that includes 1-2 layers beyond the ionic layer. This was the basis for the synthesis and characterisation of the truncated SNARE complex of this work, which presents lower thermal stability compared to the native SNARE complex but is yet able to self-assemble effectively and can be used as an immobilised protein capture system (Ferrari, E. et al. Binary Polypeptide System for Permanent and Oriented Protein Immobilization. *J. Nanobiotechnology* **2010**, *8*, 9).



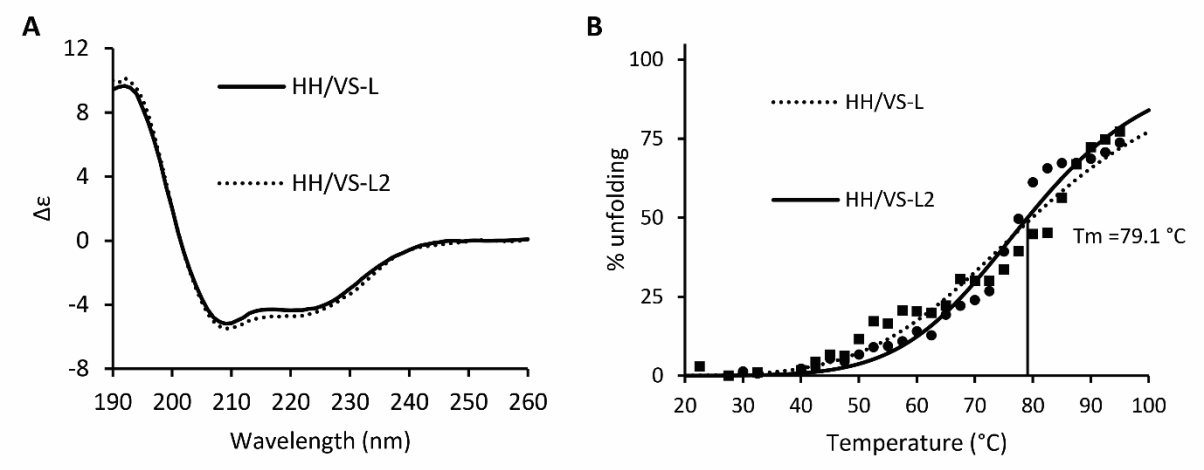

**Supplementary Figure 2**. Far-UV SRCD spectra of SNARE complexes (each protein 2 µM, pH=7.3, 20°C ), their individual components (SNAP25, Syntaxin1 and VAMP2) and partial mixtures (SNAP25/VAMP2 and SNAP25/Syntaxin1 and Syntaxin1/VAMP2). As generally known, individual SNAREs and binary mixtures are unstructured, with the exception of the SNAP25/Syntaxin pair, which form a partially structured coiled-coil as previously reported (Wiederhold, K.; Fasshauer, D. Is Assembly of the SNARE Complex Enough to Fuel Membrane Fusion? *J. Biol. Chem.* **2009**, *284*, 13143–13152).



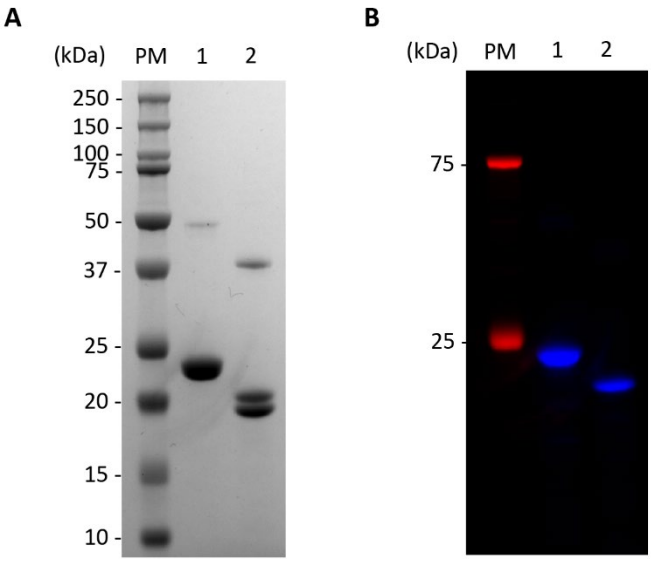

**Supplementary Figure 3**. Far-UV SRCD spectra of SNARE complexes made of SNAP25/VAMP2 and either Syntaxin 1 or Syntaxin 3 (its individual spectrum is also shown). Each protein 20 µM, pH=7.3, 20°C.



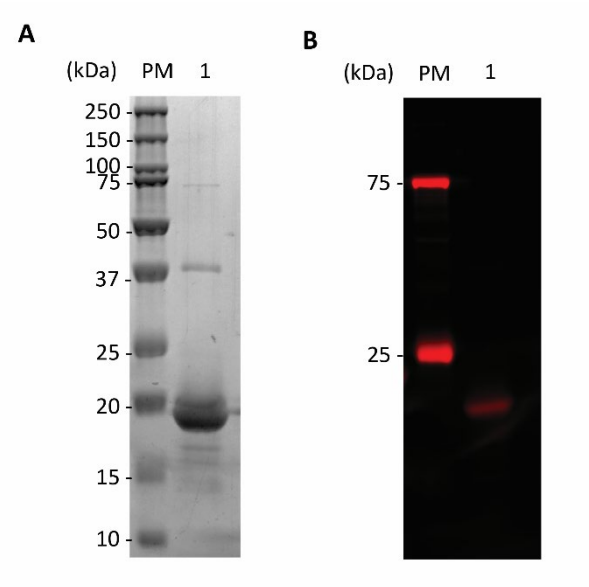

**Supplementary Figure 4**. Far-UV SRCD spectra of binary SNARE complexes and their individual components. Each protein 8 µM, pH=7.3, 20°C.



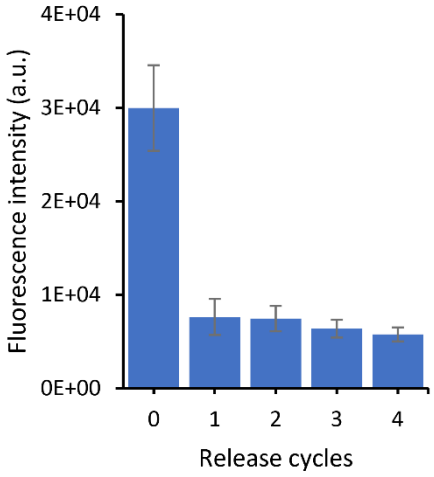

**Supplementary Figure 5**. Alternative linkers between V and S domains do not affect assembly and thermal stability properties of the binary SNARE complex formed with HH, as confirmed by (A) far-UV SRCD spectra (each protein 8 µM, pH=7.3, 20°C) and (B) SRCD temperature scan. SV-L has a linker identical to the one between the two α-helices of HH and SNAP25, whereas SV-L2 has an artificial linker of similar length made of serine and glycine residues.




**Supplementary Figure 6**. Labeling of VS-L and VS-S with Cy5-maleimide. (A) Instant blue stained SDS-PAGE of VS-L-Cy5 (lane 1) and VS-S-Cy5 (lane 2). The proportion of labeled protein migrates as a band of slightly higher molecular weight (especially visible in lane 2), revealing a conjugation efficiency of ~50%. (B) Fluorescence image of the same gel in B revealing the fluorescence of the bands with slightly higher molecular weight. Red: 600 nm filter, Blue: 700 nm filter. PM = protein marker.




**Supplementary Figure 7**. Labeling of VS-S with Cy3-maleimide. (A) Instant blue stained SDS-PAGE of VS-S-Cy3 (lane 1). The proportion of labeled protein migrates as a band of slightly higher molecular weight, revealing a conjugation efficiency of less than 50%. (B) Fluorescence image (600 nm filter) of the same gel in B revealing the fluorescence of the bands with slightly higher molecular weight. PM = protein marker.




**Supplementary Figure 8**. Fluorescence intensity of captured VS-L-Cy5 after Sepharose-GST-HH was heated to 80°C and cooled to 20°C for 0 to 4 cycles. Average of three measurements, error bars represent the standard deviation.