

SUPPLEMENTARY INFORMATION

Design of a Reversible Inversed pH-Responsive Caged-Like Protein

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The secondary structure contents of E2-WT and E2-GALA at different pH-s (Table S1) were calculated from the corresponding CD spectra using CDNN program.¹ The results indicate the trends of secondary structure change of E2-WT or E2GALA at different pH-s.

Table S1. Comparison of Secondary Structure Proportions of E2-WT and E2-GALA at different pH-s.

	E2-WT			E2-GALA		
	pH 7.0	pH 5.0	pH 4.0	pH 7.0	pH 5.0	pH 4.0
α -helix	39%	37%	37%	34%	35%	32%
β -sheet	19%	19%	19%	19%	20%	20%
Random Coil	24%	25%	26%	27%	26%	29%

Other mutant E2 proteins with different lengths of GALA peptides and different substitution sites are also designed (Table S2). Size measurements using DLS were considered as primary evidence to

indicate the self-assembly status. By incorporating GALA peptides, the interactions between trimer structures are expected to be disrupted. Almost all GALA-incorporated E2 proteins (E2-GALA, E2-GALA2, and E2-GALA3) are present as trimers at pH 7.0, except for the one with longer length of GALA peptide (E2-GALA4); E2-GALA4 was not produced in soluble form and was excluded from subsequent studies.

At pH 5.0, E2-GALA2 (with 2 more amino acids at the C-terminus than E2-GALA) and E2-GALA3 (lacking D419 and E421) are present in small diameters which are comparable to the sizes of trimer or monomer structures of E2 protein. However, when the pH is lowered to 4.0, both E2-GALA2 and E2-GALA3 show significant increase in diameters one order of magnitude, indicating the formation of random aggregates and failure to form correctly self-assembled cages.

Table S2. Summary of different designs of GALA peptide incorporated at the C-terminus of E2 proteins and their hydrodynamic sizes (nm) at different pH-s.

E2 variants	GALA unit (WT sequences are in black bolded fonts)	pH 7.0	pH 5.0	pH 4.0
E2-WT	419- DPELLLMEA	25.04 ± 0.63	28.01 ± 1.51	26.83 ± 1.13
E2-GALA	419- DPE <i>AALAEALA</i>	8.05 ± 0.19	8.12 ± 0.16	24.32 ± 0.43
E2-GALA 2	419- DPE <i>AALAEALAEA</i>	9.42 ± 0.76	9.33 ± 0.92	119.4 ± 75.6
E2-GALA 3	419- <i>LAEALAEA</i>	7.78 ± 0.16	8.41 ± 0.32	298.5 ± 89
E2-GALA 4	419- <i>LAEALAEHLAEALAE</i>	no soluble expression		
E2-ΔC9	Amino acids 419-427 are truncated	8.01 ± 0.21	7.99 ± 0.33	8.23 ± 0.18

Molecular dynamic simulations video on the folding of the GALA peptide is available online.

References

- (1) Bohm, G.; Muhr, R.; Jaenicke, R., *Protein Eng* **1992**, 5, 191-195.