Supplementary Material (ESI) for Soft Matter

Electronic Supplementary Information

Controlled Self-Assembly of α-Helix-Decorated Peptide Nanostructures

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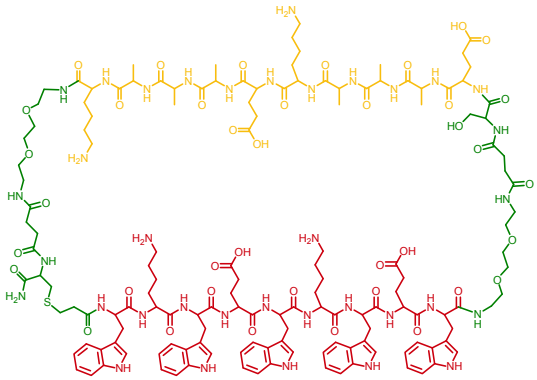
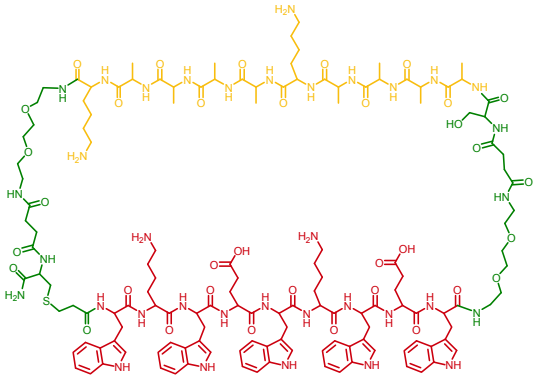
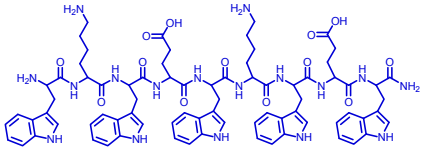


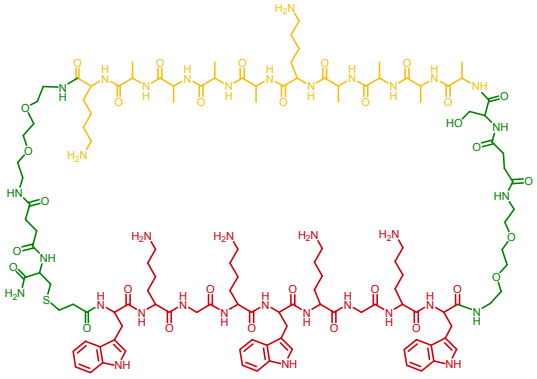
PEP-1



PEP-3



PEP-2



PEP-4

Figure S1 Peptide building block structures.

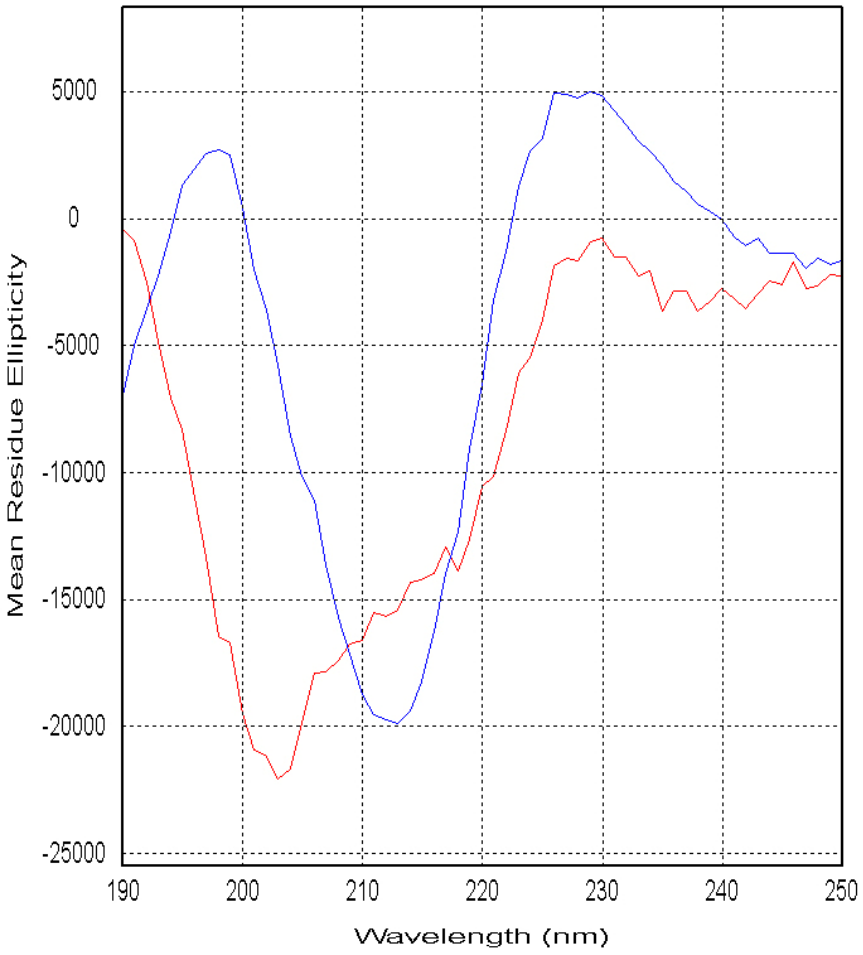


Figure S2 Effect of acetonitrile (ACN) on the β-sheet-mediated self-assembly of PEP-2. CD spectrum of PEP-2 in water (blue) and in water:ACN (1:1, red).

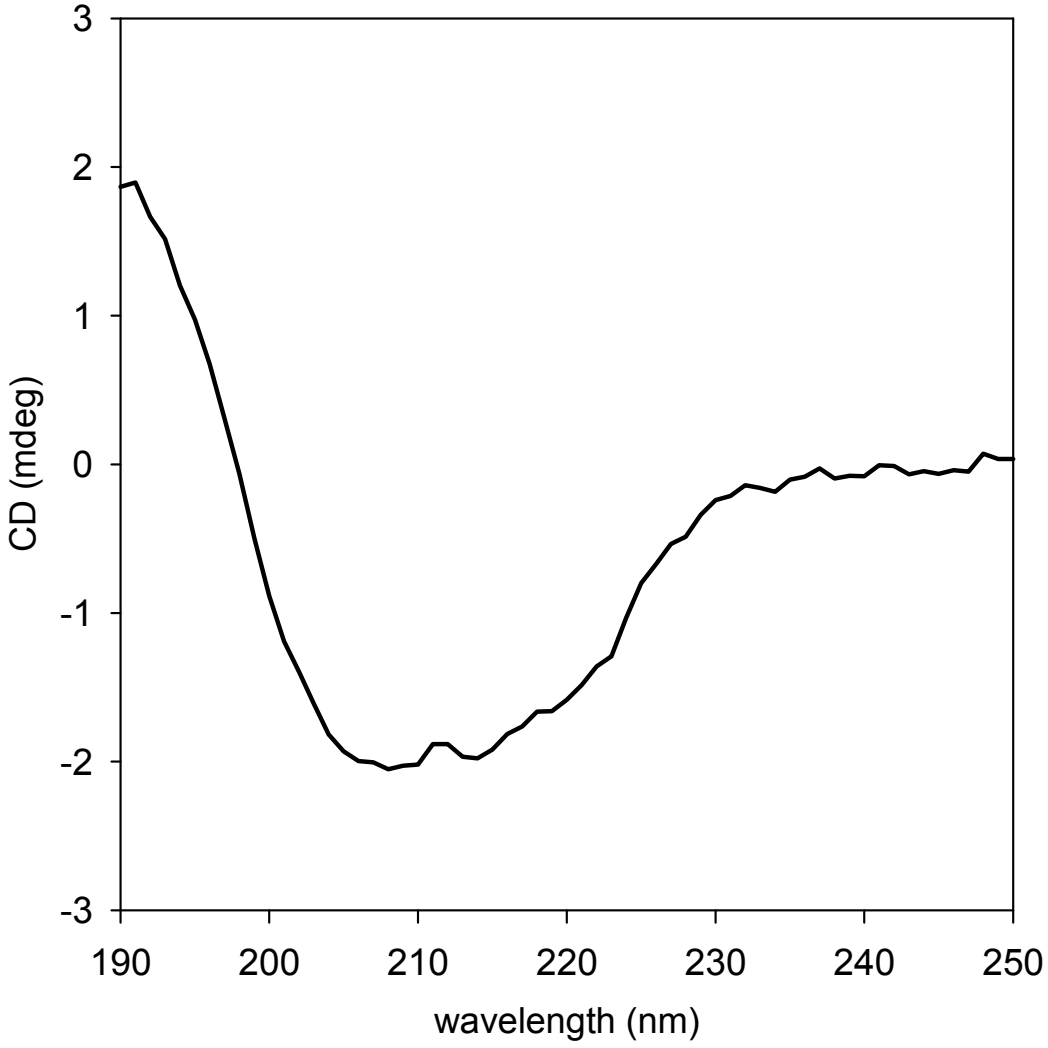


Figure S3 CD spectrum of PEP-1 & PEP-2 coassembly.

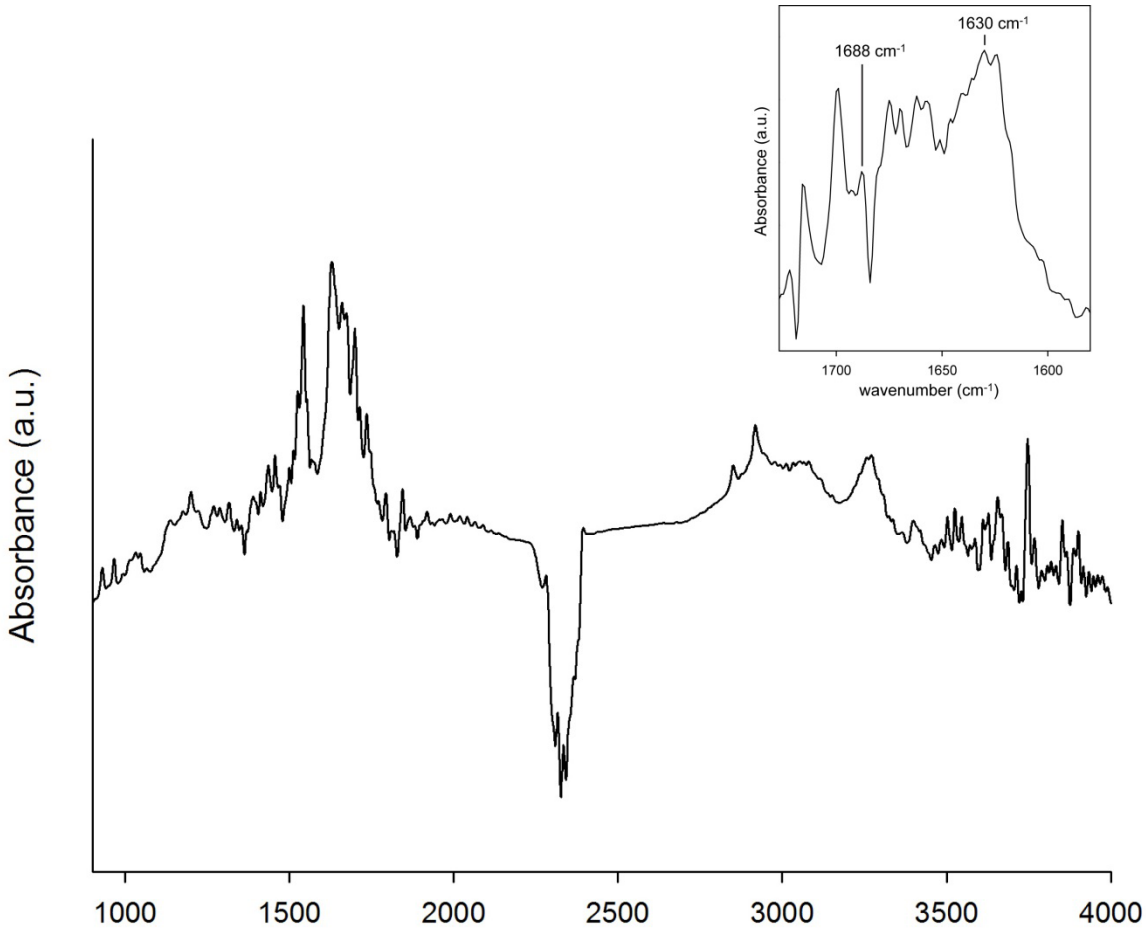


Figure S4 FTIR spectrum of PEP-1 and PEP-2 coassembly. Inset: enlarged amide region.

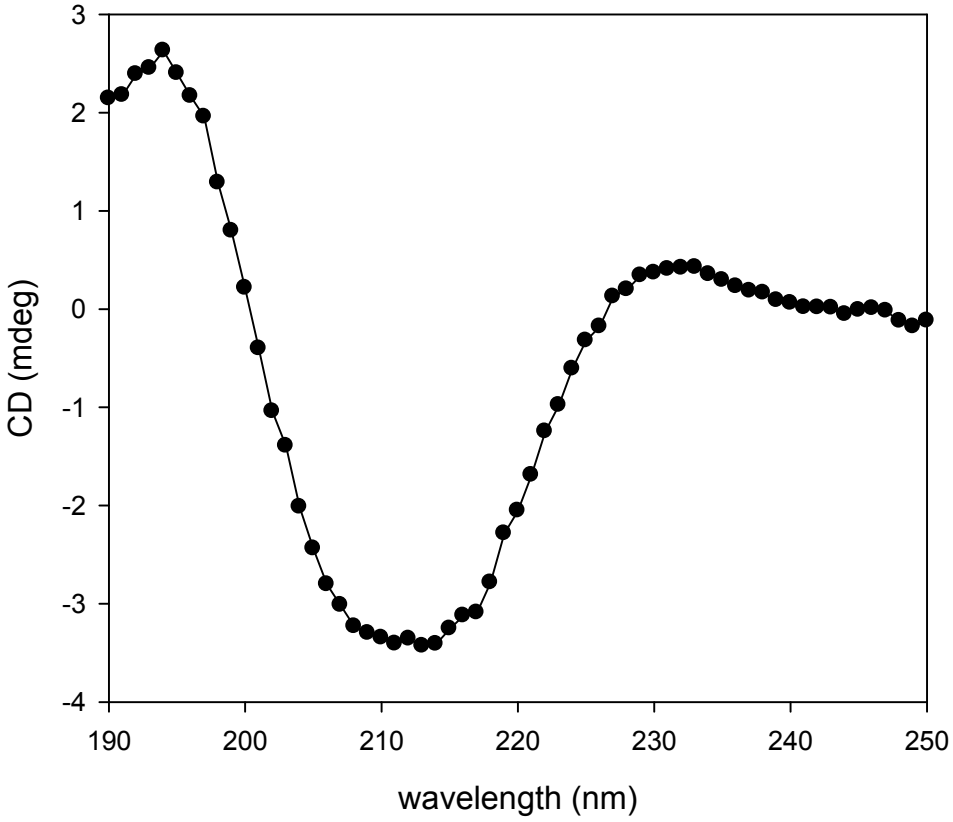


Figure S5 Effects of acetonitrile evaporation rate on the coassembly of PEP-1 and PEP-2. In this sample, acetonitrile was rapidly evaporated (< 20 min) by using a centrifugal vacuum concentrator.

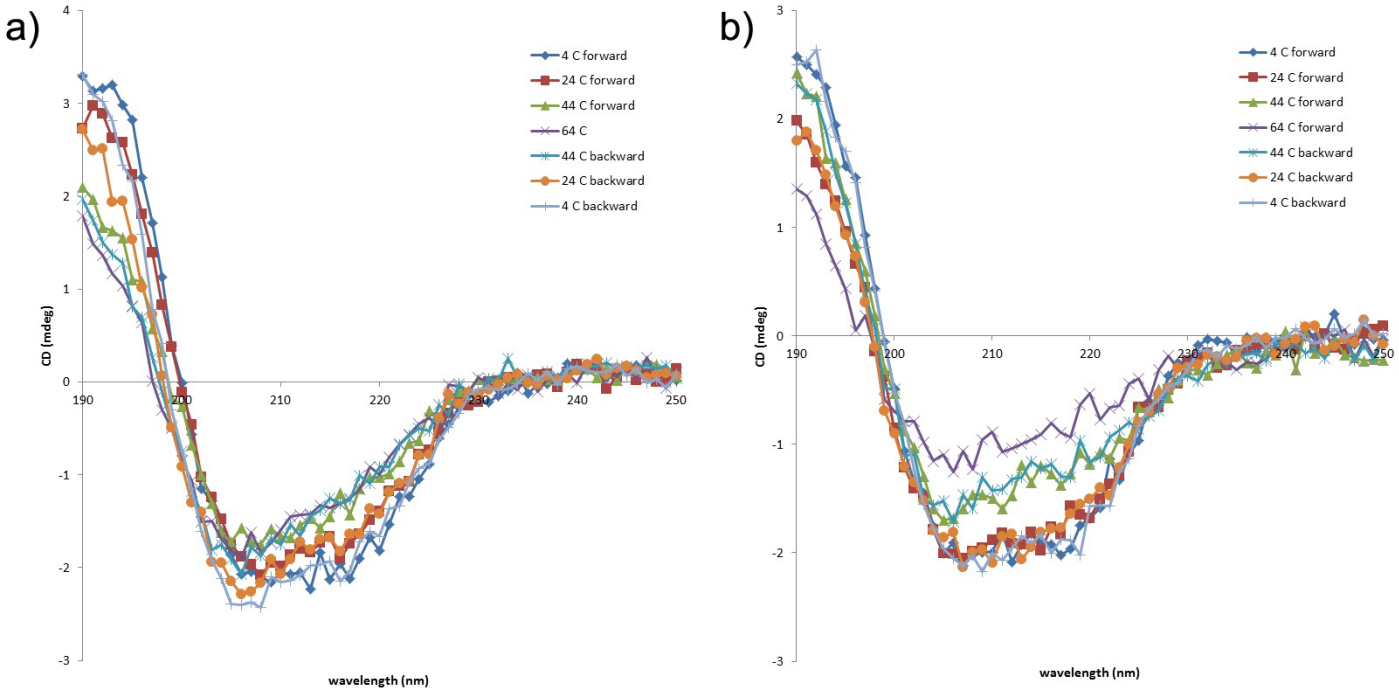


Figure S6 Effects of temperature on the stability of a) PEP-1 and b) PEP-1 and PEP-2 coassembly.

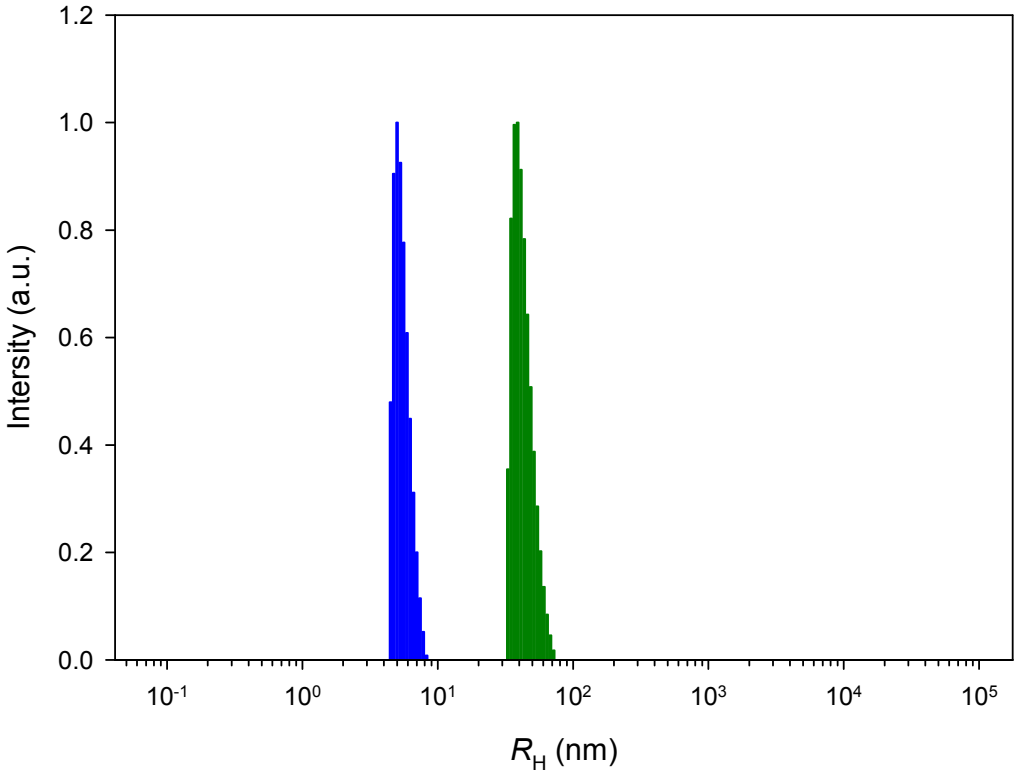


Figure S7 Dynamic light scattering data for PEP-1 nanostructures (blue) and the coassembled nanostructures of PEP-1 and PEP-2 (green).

Materials and Methods

Synthesis. Peptide was synthesized on Rink Amide MBHA resin LL (Novabiochem) using standard Fmoc protocols on a Tribute™ peptide synthesizer (Protein Technologies, Inc). Standard amino acid protecting groups were employed except cysteine, in which an acid-labile methoxytrityl (Mmt) group was used. The oligoethylene glycol-based linker, *N*-(Fmoc-8-amino-3,6-dioxaoctyl)succinamic acid (Fmoc-PEG_2-Suc-OH), was purchased from Anaspec. The peptide-attached resin (20 μmol of N-terminal amine groups) was swollen in *N*-methyl-2-pyrrolidone (NMP) for 30 min. For cyclization, bromoacetic acid was first coupled to the N-terminal part of the resin-bound peptide. Before addition to the resin, a mixture of bromoacetic acid (28 mg, 200 μmol) and *N*,*N'*-diisopropylcarbodiimide (15.5 μL, 100 μmol) in NMP was incubated for 10 min for carboxyl activation. The reaction was continued for 1 h with shaking at room temperature, in a 6 mL polypropylene tube with a frit (Restek). The resin was then washed successively with NMP and dichloromethane (DCM). For orthogonal deprotection of the Mmt group from the cysteine, the resin was treated with 1% trifluoroacetic acid (TFA) in DCM several times (1 min × ~5). Intramolecular cyclization reaction was performed in 3 mL of 1% diisopropylethylamine (DIPEA) in NMP overnight with shaking at room temperature. The resin was then successively washed with NMP and acetonitrile, and dried *in vacuo*. The dried resin was treated with cleavage cocktail (TFA：1,2-ethanedithiol：thioanisole; 95 : 2.5 : 2.5) for 3 h, and was triturated with *tert*-butyl methyl ether. The peptides were purified by reverse-phase HPLC (water–acetonitrile with 0.1% TFA). The molecular weight was confirmed by MALDI-TOF mass spectrometry. The purity of the peptides was >95% as

determined by analytical HPLC. Concentration was determined spectrophotometrically in 8 M urea using a molar extinction coefficient of tryptophan (5,500 $M^{-1}cm^{-1}$) at 280 nm.

Circular dichroism. CD spectra were measured using a Chirascan™ Circular Dichroism Spectrometer equipped with peltier temperature controller (Applied Photophysics., Ltd). Spectra were recorded from 250 nm to 190 nm using a 2 mm path-length cuvette. Scans were repeated five times and averaged. Molar ellipticity was calculated per amino acid residue. Peptide concentration was 5 μM. Peptides were typically prepared in 20 mM KF solution unless described otherwise. Sample solutions were incubated at least for 2 days before measurement, and essentially the same CD spectra were obtained after prolonged incubation, indicating thermodynamic equilibrium states.

Transmission electron microscopy. Three μL of sample was placed onto a carbon-coated copper grid and dried completely. Then 2 μL of 1 % (w/v) uranyl acetate solution was added for 1 min and excess solution was wicked off by filter paper. Sample concentrations were typically 5 - 20 μM in 20 mM KF. The specimen was observed with a JEOL-JEM 2010 instrument operating at 100 kV.

Infrared spectroscopy. For FT-IR measurement, 300 μL of the sample (50 μM in 20 mM KF) was cast from the solution onto ZnSe window. Twenty thousand scans were acquired on a Bruker Vertex 70 FT-IR spectrometer.

Dynamic light scattering (DLS). DLS experiment was performed at room temperature with LV/CGS-3 Compact Goniometer System equipped with He-Ne laser operating at 632.8 nm. The scattering angle was 90°. Before measurement, the sample was centrifuged at 16,110 × g for 20 min to sediment any dust particles. Sample concentrations were typically 5 - 20 μM in 20 mM KF. The size distribution was determined by using a constrained regularization method.