Electronic Supporting Information (ESI)

Aggregation Propensity of Therapeutic Fibrin-Homing Pentapeptides: Insights from Experiments and Molecular Dynamics Simulations

David Zanuy,[a*] *Anna Puigalí-Jou,*[a] *Paolo Conflitti,*[b†], *Gianfranco Bocchinfuso,*[b] *Antonio Palleschi*[b] *Carlos Alemán*[a,c]

[a] Department of Chemical Engineering and Barcelona Research Center in Multiscale Science and Engineering, Universitat Polytècnica de Catalunya, 08019 Barcelona, Spain
[b] Dipartimento di Scienze e Tecnologie Chimiche, Universita`di Roma‘‘Tor Vergata’’, via della ricerca scientifica 1, 00133, Rome, Italy
[c] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10-12, 08028 Barcelona Spain
[†] Currently at Università della Svizzera italiana (USI), Faculty of Biomedical Sciences, Institute of Computational Science, Via G. Buffi 13, CH-6900 Lugano, Switzerland

Correspondence: david.zanuy@upc.edu and carlos.aleman@upc.edu

Table S1.- Simulation conditions of all simulated systems. First three simulations were discarded since no aggregation event was detected in the simulated timeframe.

Simulation	a × b × c (nm)	# water molecules	[peptide] mg/ml	Assembly event simulated time
MD0-1	22 21 21	324567	1.6	None (100 ns)
MD0-2	22 15 15	165658	3.1	None (100 ns)
MD0-2	20 12 12	96367	5.2	None (100 ns)
MD I	9.5 8.5 9.0	23779	20.8	Yes (60 ns)
MD II	7.5 7.5 7.5	13579	35.8	Yes (60 ns)
MD III	6.5 6.5 6.5	8653	55.1	Yes (110ns)

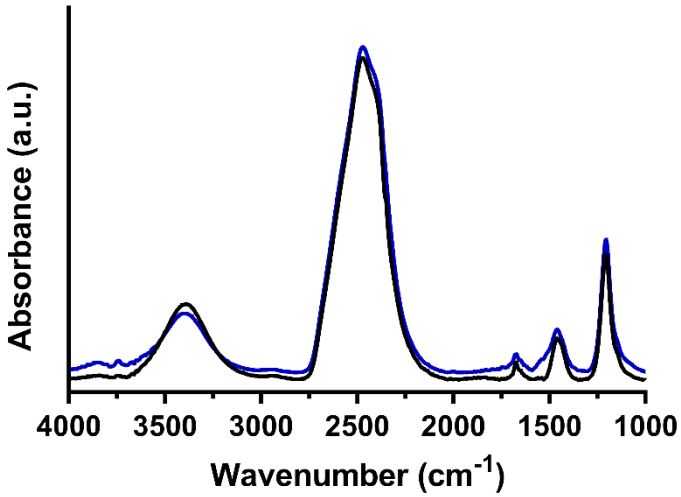

Figure S1.- Full FTIR spectra of CREKA (Black line) and CRMeEKA (blue line).

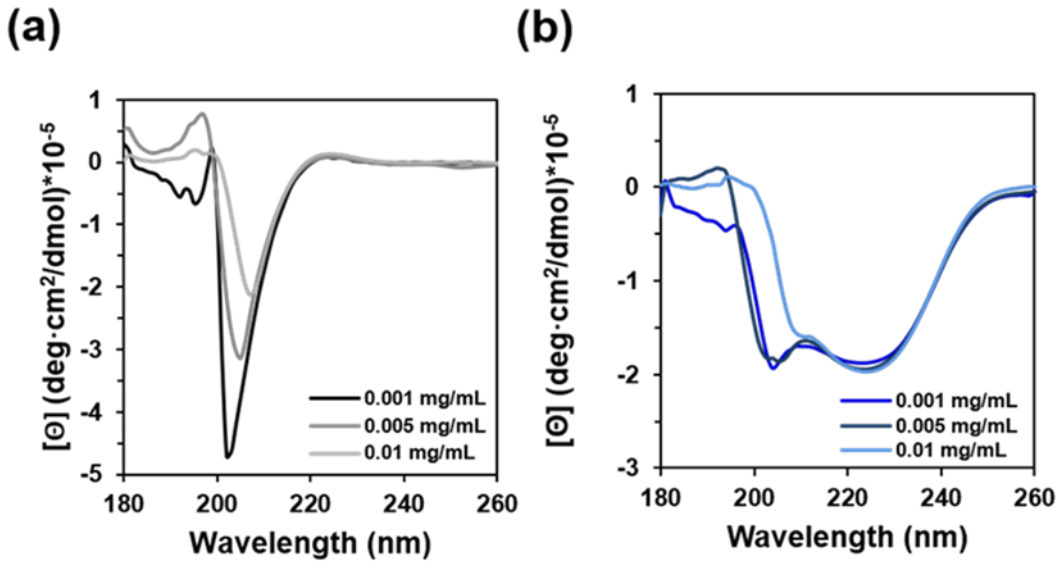

Figure S2.- CD spectra for CREKA **(a)** and CRMeEKA **(b)** in PBS 1x pH 7.4 at different peptide concentrations

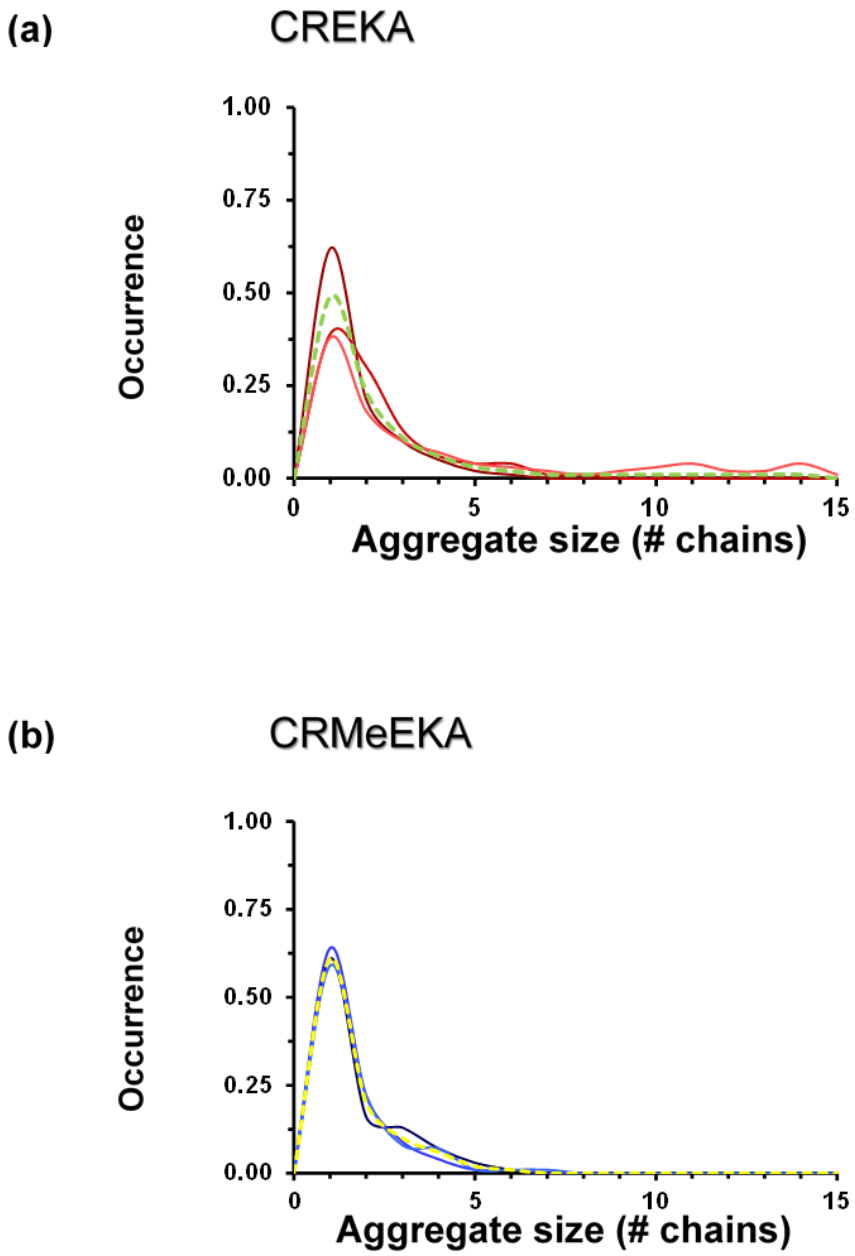

Figure S3.- Cluster size occurrence versus the different studied time frames. **(a) CREKA** aggregates. Solid dark red line represents time frame 0-70ns, solid red line represents time frame 70-130 ns, solid light red line represents time frame 130-230 ns, dashed orange line encompasses all 230 ns of simulation **(b) CREMeKA** Solid dark blue line represents time frame 0-70ns, solid blue line represents time frame 70-130 ns, solid light blue line represents time frame 130-230 ns, dashed yellow line encompasses all 230 ns of simulation.

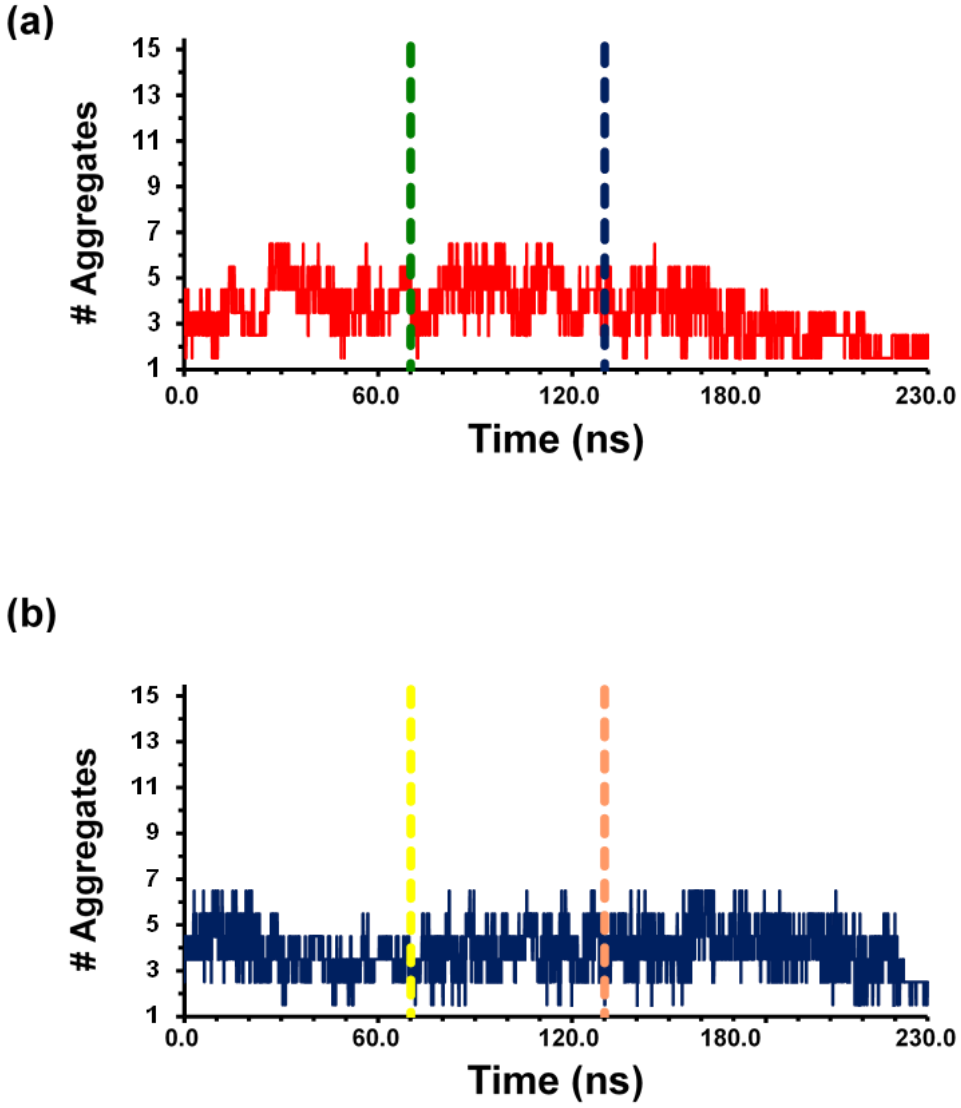

Figure S4.- Number of aggregates versus time for CREKA **(a)** and CRMeEKA **(b)**. Changes in box size are indicated with dashed vertical lines.

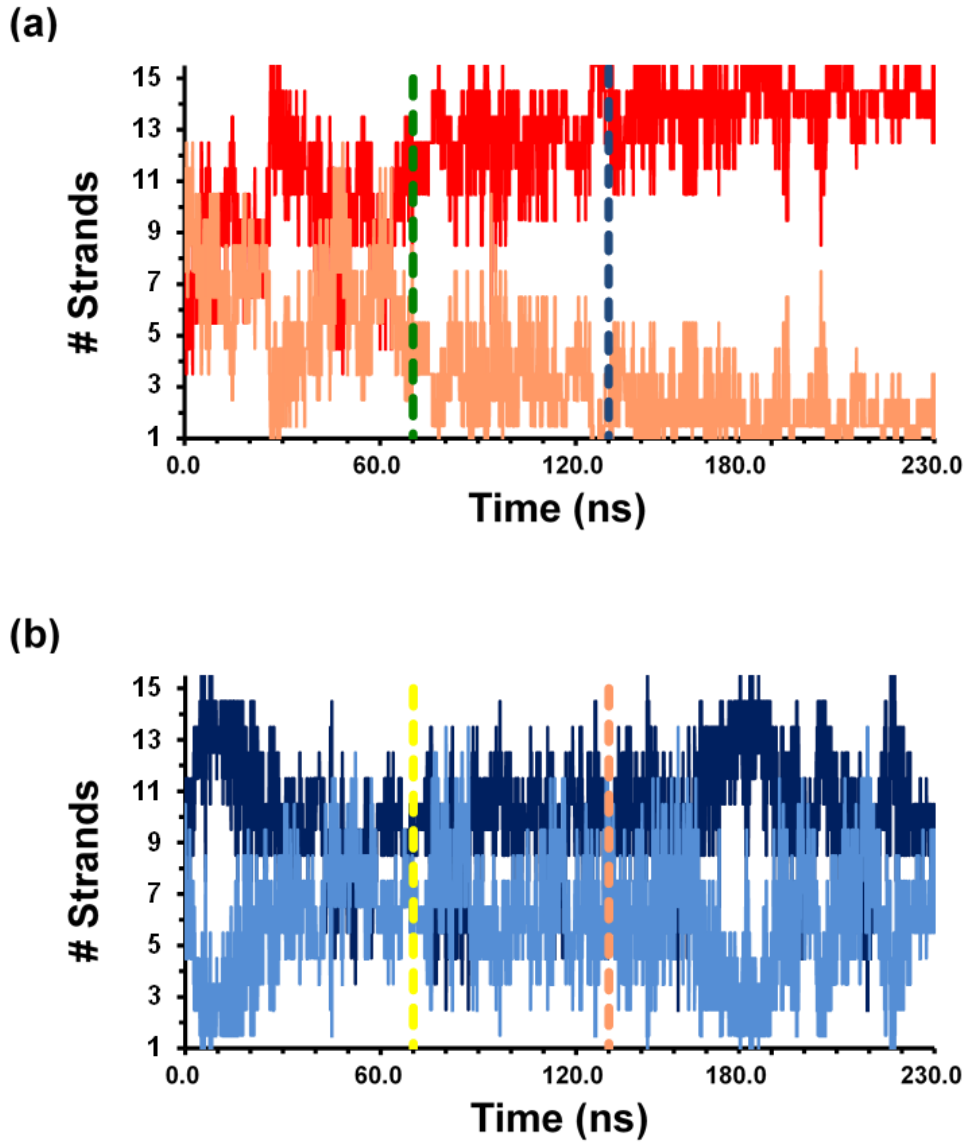

Figure S5.- Number of chains present in aggregates and of single chains present versus time for CREKA **(a)** where red line indicates strands in assemblies and orange line indicates single chains and CRMeEKA **(b)** where dark blue line indicates strands in assemblies and light blue line indicates single chains

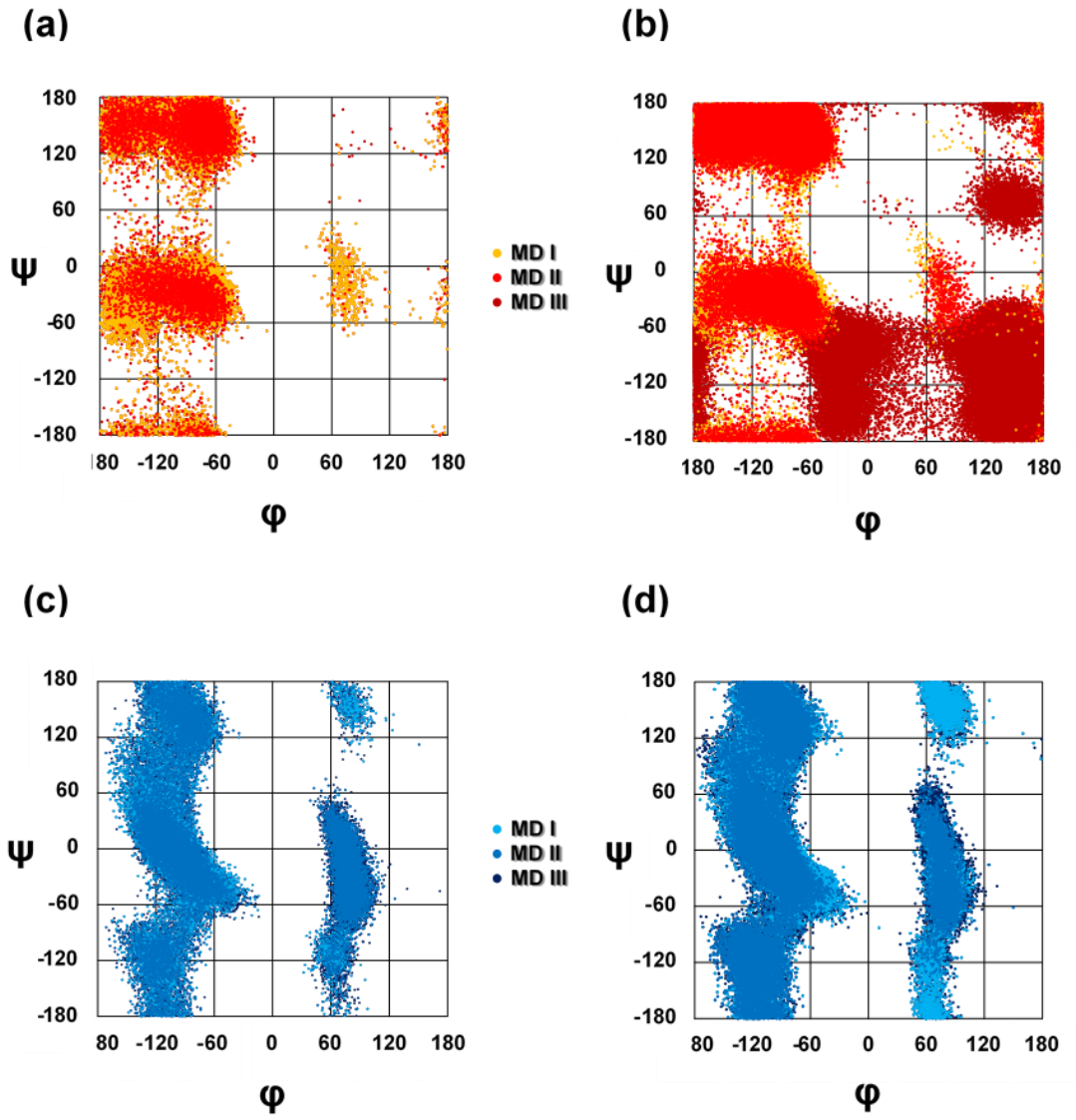

Figure S6.- Accumulated Ramachandran plot of the central residue for: **(a)** Glu residue in CREKA strands not forming aggregates **(b)** Glu residue in CREKA strands forming aggregates **(c)** Met-Glu residue in CRMeEKA strands not forming aggregates **(d)** Met-Glu residue in CRMeEKA strands forming aggregates.

(!) Colour code is explained in offset charts.

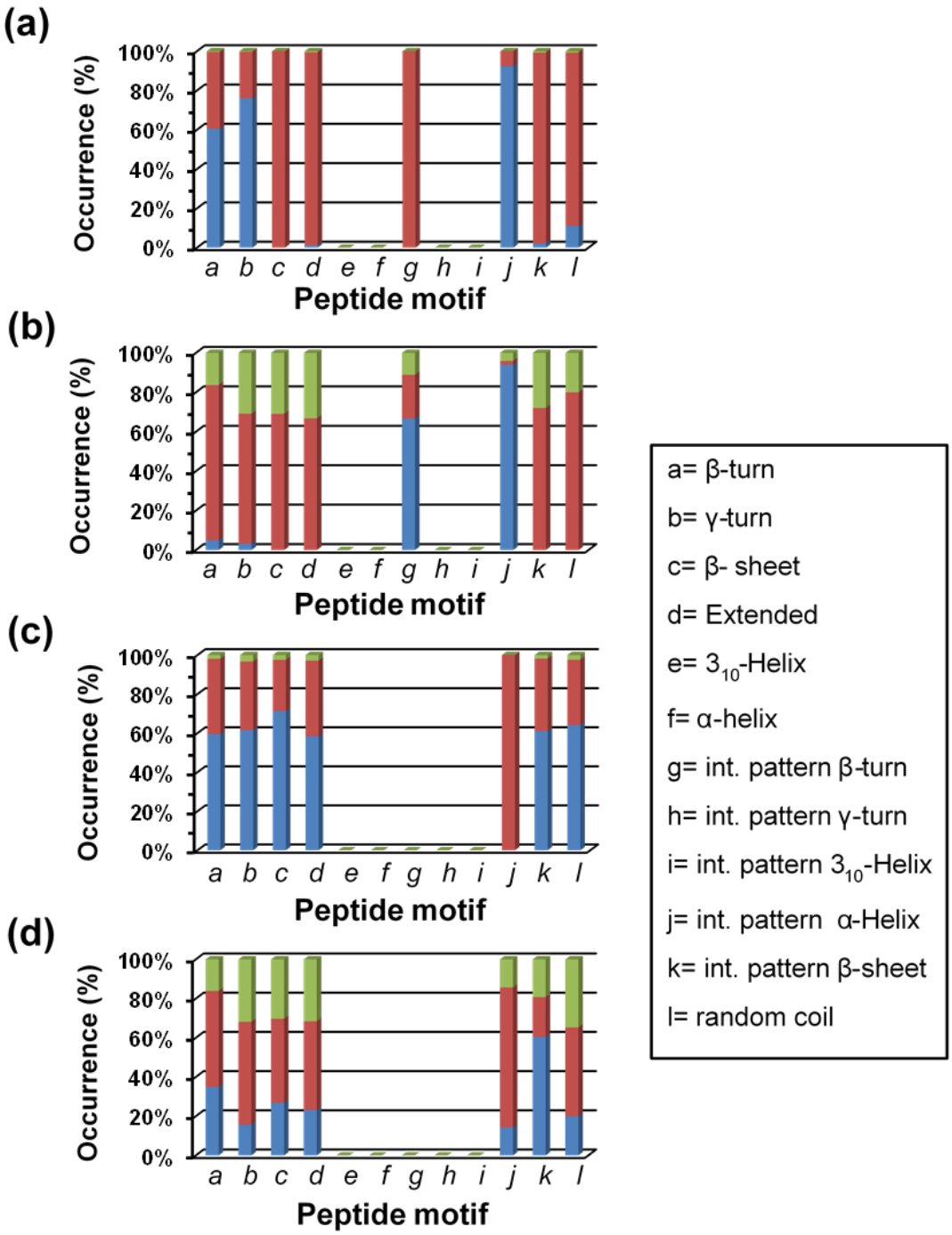

Figure S7.- Correlation between CREKA conformations and interaction types. Green color corresponds to side chain side chain interactions, red to side chain main chain interactions and blue main chain interactions. **(a)** intra-strand Hydrogen Bonds **(b)** intra-strand Salt-Bridges **(c)** inter-strand Hydrogen Bonds **(d)** inter-strand Salt-Bridges. Legend box indicates all kinds of examined conformational motifs.

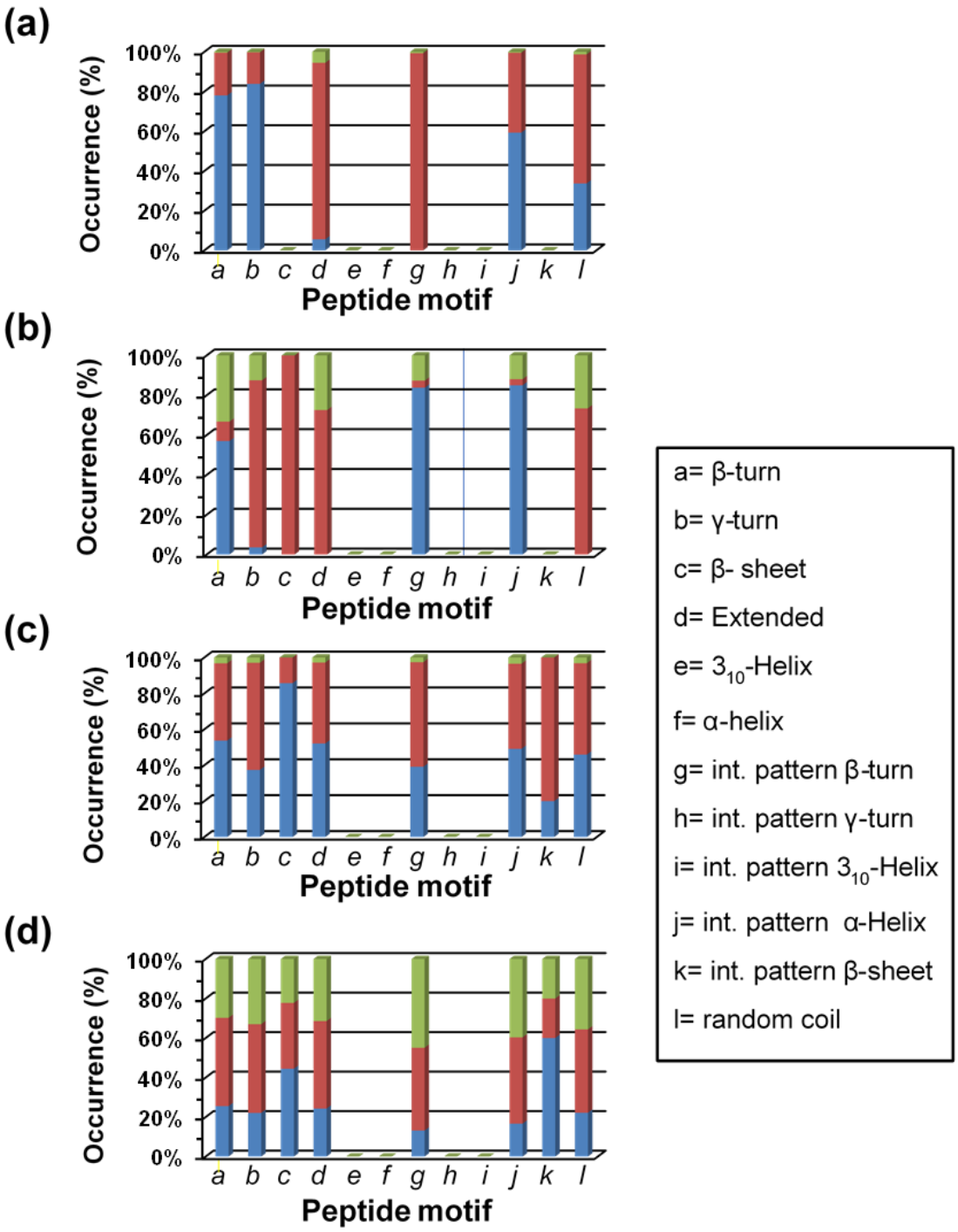

Figure S8.- Correlation between CRMeEKA conformations and interaction types. Green color corresponds to side chain side chain interactions, red to side chain main chain interactions and blue main chain interactions. **(a)** intra-strand Hydrogen Bonds **(b)** intra-strand Salt-Bridges **(c)** inter-strand Hydrogen Bonds **(d)** inter-strand Salt-Bridges. Legend box indicates all kinds of examined conformational motifs.

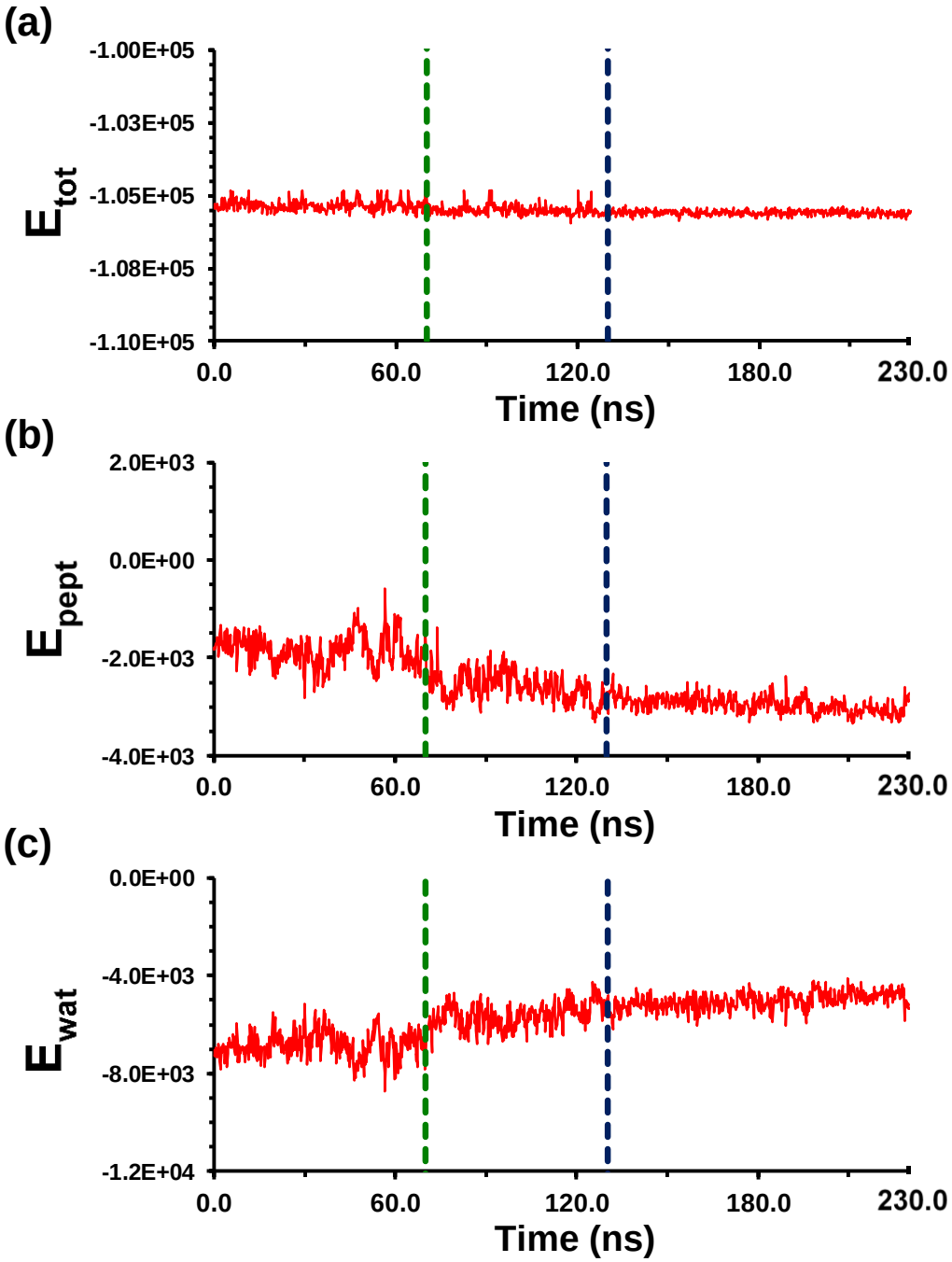

Figure S9.- CREKA and their internal energy components (kcal·mol^{-1}·strand^{-1}) versus simulated time. From top to bottom: **(a)** Total interaction energy (E_{tot}) **(b)** Peptide - Peptide (E_{pept}) **(c)** Peptide – solvent (E_{wat}).

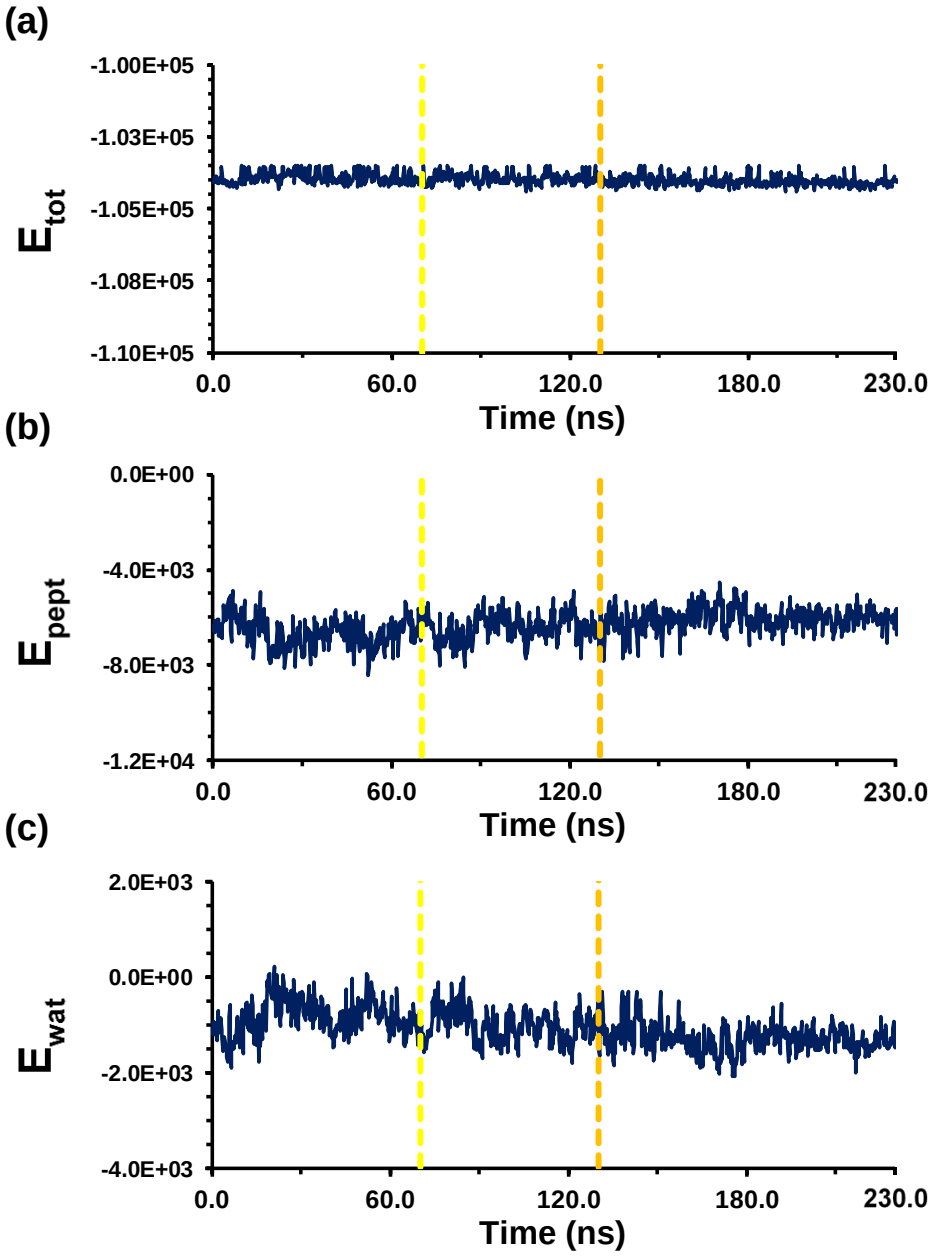

Figure S10.- CREMeKA and their internal energy components (kcal·mol^{-1}·strand^{-1}) versus simulated time. From top to bottom: **(a)** Total interaction energy (E_{tot}) **(b)** Peptide - Peptide (E_{pept}) **(c)** Peptide – solvent (E_{wat}).

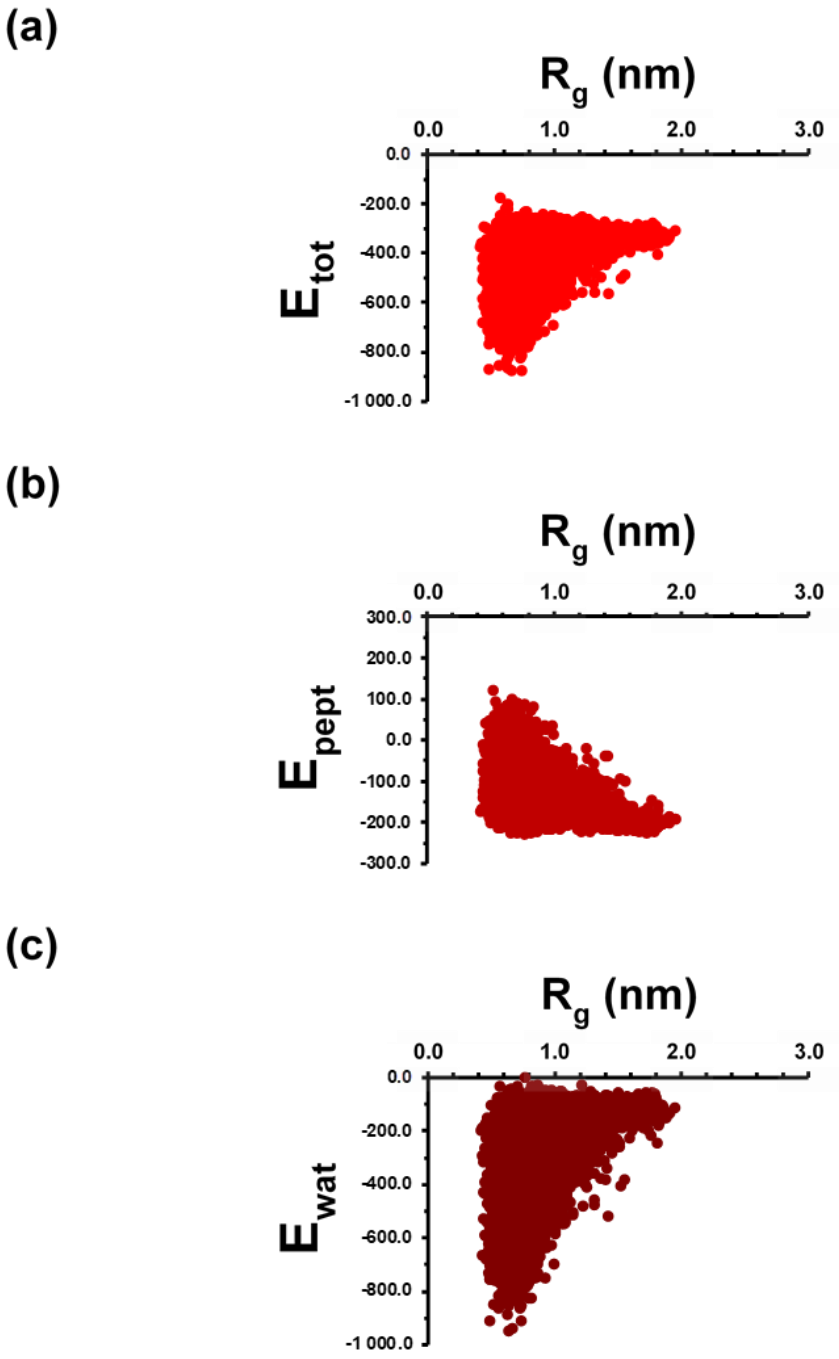

Figure S11.- Correlation between the radius of gyration of lonely strands and the strands assemblies of CREKA and their internal energy components (kcal·mol^{-1}·strand^{-1}). From top to bottom, the radius of gyration versus: **(a)** Total interaction energy (E_{tot}) **(b)** Peptide - Peptide (E_{pept}) **(c)** Peptide – solvent (E_{wat}).

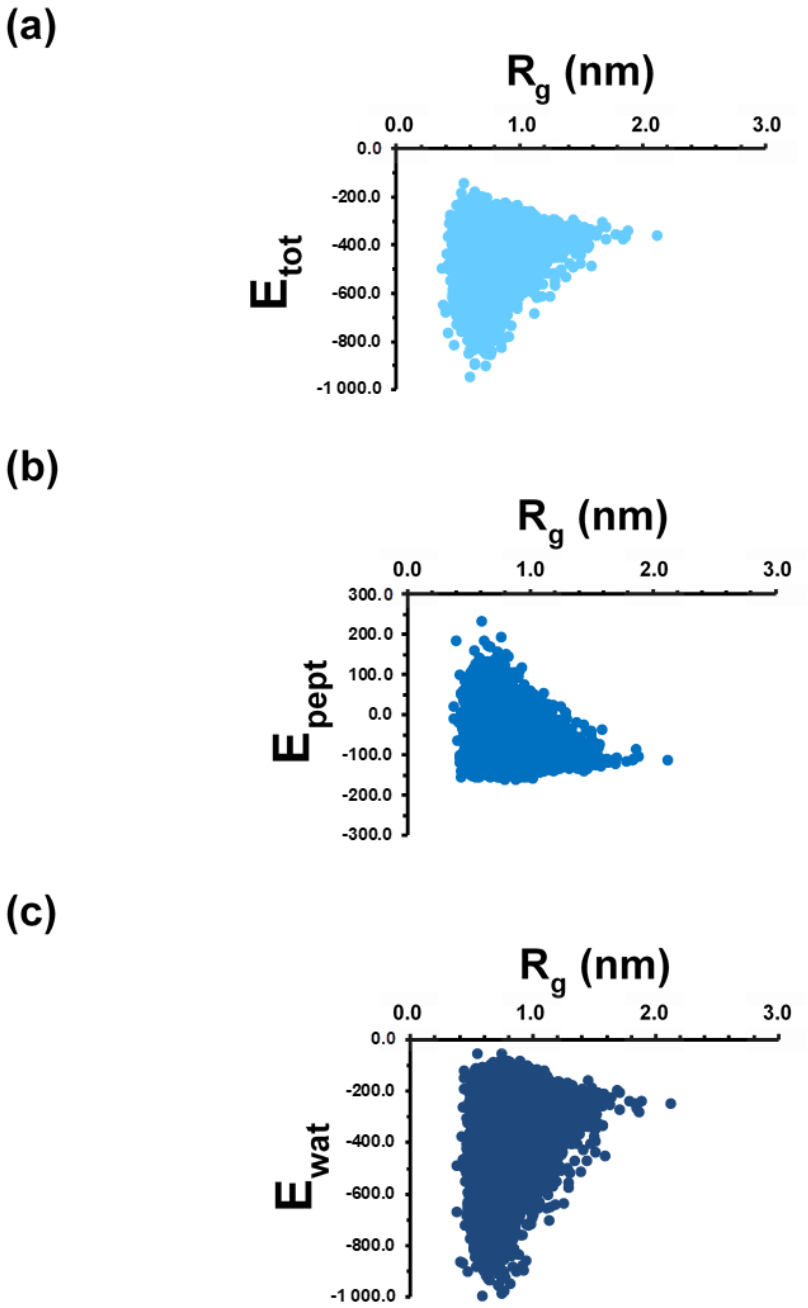

Figure S12.- Correlation between the radius of gyration of lonely strands and the strands assemblies of CRMeEKA and their internal energy components (kcal·mol^{-1}·strand^{-1}). From top to bottom, the radius of gyration versus: **(a)** Total interaction energy (E_{tot}) **(b)** Peptide - Peptide (E_{pept}) **(c)** Peptide – solvent (E_{wat}).