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Online Supplementary Data

FOLDING AMPHIPATHIC HELICES INTO MEMBRANES: AMPHIPHILICITY TRUMPS HYDROPHOBICITY

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Folding of the $A_8Q_3L_4$ family of peptides in buffer

In order to calculate the free energies of folding of the $A_8Q_3L_4$ family of peptides in water, we used the alcohol-induced α-helix formation method of Hirota *et al.*[1,2] We approximated the alcohol-induced transition by a two-state mechanism, *i.e.*, the only states present are the native and the α-helical states for all the peptides. The CD spectra of the titrations of peptides with 2,2,2-trifluoroethanol (TFE) showed an isodichroic point at ~ 203 nm (**Fig. S1**), consistent with a two-state approximation.

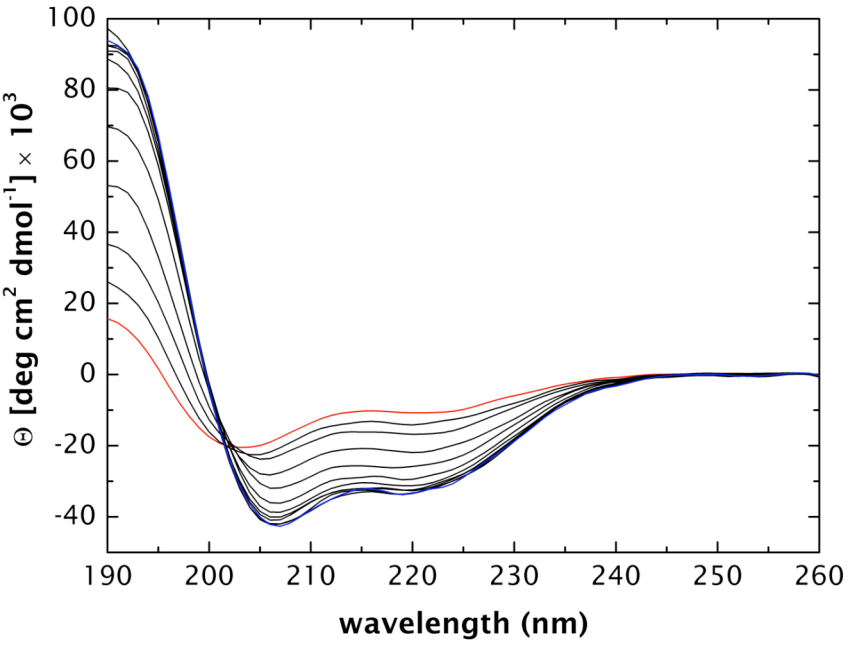

Figure S1. CD spectra of $A_8Q_3L_4$-4.72 in buffer with increasing additions of trifluoroethanol (TFE), ranging from 0.0 M (red curve) to 5.5 M (blue curve). Notice the isodichroic point at ~203 nm.

Analysis of two-state alcohol-induced folding of unfolded or partially folded peptides[3] follows the same methods used for analyzing the two-state denaturation of soluble proteins using a chaotropic agent, such as urea. A good description of the use of denaturation for studying the stability of soluble proteins is given by Goldenberg[4]. The parameter we follow for alcohol-induced folding is the molar ellipticity at 222 nm, $[\Theta]_{222}$. Plots of $[\Theta]_{222}$ against alcohol concentration follows a Boltzmann distribution, as shown below. A side benefit of this method is that one can estimate the ellipticities

for fully folded and fully unfolded peptides, Θ_H and Θ_{RC}, respectively, which are necessary for determining the fractional ellipticities f_α using eq. (6).

The free energy change ΔG_0 for folding in the absence of TFE is given by $\Delta G_0 = -RT\ln K_0 \equiv \Delta G_{AC}$, where $K_0 = f_\alpha(0)/f_u(0)$ (see **Table S1**, below). The terms $f_\alpha(0)$ and $f_u(0)$ indicate the fractions of folded and unfolded peptide, respectively, for [TFE] = 0.

For [TFE] ≠ 0, the free energy of folding ΔG_F can be described[2,3] by

$$\Delta G_F = \Delta G_0 - m[\mathrm{TFE}] \tag{S1}$$

where m measures the dependence of ΔG_F on TFE concentration. Rearrangement of eq. S1 after substitution of the definitions of ΔG_F and ΔG_0 yields

$$K_F = K_0 \exp(m[\mathrm{TFE}]/RT) \tag{S2}$$

It follows from $K_F = f_\alpha/(1-f_\alpha)$ that

$$f_\alpha = K_F/(1+K_F) = 1/(1+K_F^{-1}) \tag{S3}$$

Substitution of eq. S2 into eq. S3 yields

$$f_\alpha = 1/(1 + K_0^{-1} \exp(-m[\mathrm{TFE}]/RT)) \tag{S4}$$

Eq. S4 is the Boltzmann function. One can readily show that $f_\alpha = f_\alpha(0)$ for [TFE] = 0, $f_\alpha \rightarrow 1$ as [TFE] becomes large and positive, and $f_\alpha \rightarrow 0$ as [TFE] becomes large and negative. Of course, [TFE] cannot be less than 0, but for the purpose of curve fitting that does not matter. Because S4 is the Boltzmann function, one can fit molar ellipticity data $[\Theta]_{222}([\mathrm{TFE}])$ to a general Boltzmann fitting function using non-linear least squares methods. Defining [TFE] = c, Θ_H and Θ_{RC} can be determined from

$$[\Theta]_{222} = \frac{\Theta_{RC} - \Theta_H}{1 + \exp\left(c/\Delta c\right)} + \Theta_H \tag{S5}$$

where Δc is a parameter that describes the width of the transition from f_u to f_α as the TFE concentration is increased.

Each peptide of the family was titrated with TFE and methanol (data not shown) at T = 298 K, and the resulting data fitted to eq. S5. The results are shown in **Fig. S2**.

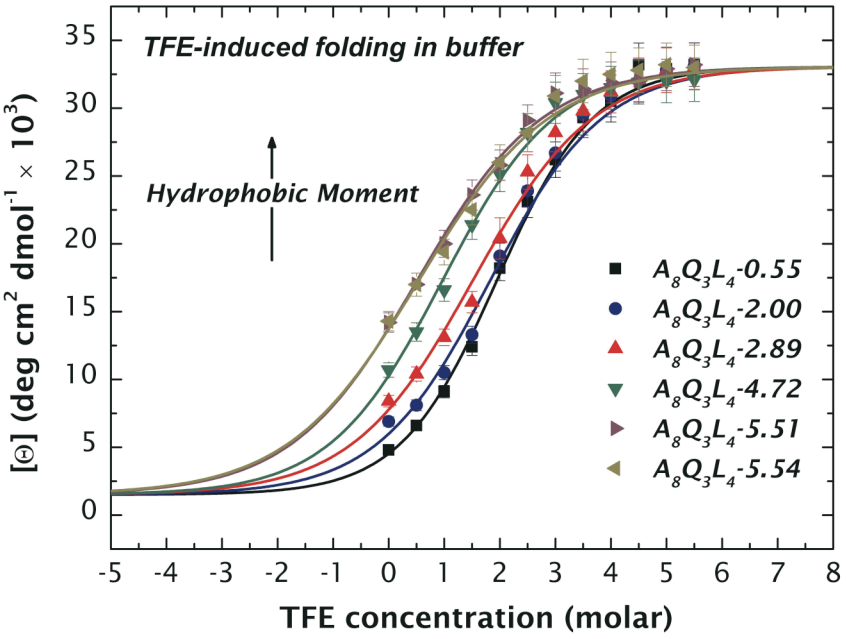

Figure S2. Plots of molar ellipticity versus TFE concentration for the $A_8Q_3L_4$ family of peptides. Notice that all peptides are maximally folded in ≈ 6 M TFE. From these data, we established that Θ_{RC} = -1500 and Θ_H = -33050 deg cm^2 dmol^{-1}. Because Θ_H is the same for all of the peptides, we assume that Θ_H corresponds to 100% helicity. This assumption is supported by the comprehensive data and analysis of Chen et al.[5]. Their Equation (2) used with parameters from their Table IV yield a theoretical value of Θ_H = -33529 deg cm^2 dmol^{-1}, which is satisfyingly close to our value.

From these data, we obtained values of ΔG_{AC} and $f_\alpha(0)$, which are summarized in **Table S1**.

Table S1. Helicities and folding free energies (ΔG_{AC}) in buffer for the $A_8Q_3L_4$ family of peptides and for melittin. The free energy change ΔG_0 for folding in the absence of TFE is given by $\Delta G_0 = -RT\ln K_0 \equiv \Delta G_{AC}$, where $K_0 = f_\alpha(0)/f_u(0)$. The terms $f_\alpha(0)$ and $f_u(0)$ indicate the fractions of folded and unfolded peptide, respectively, for [TFE] = 0 M.

[a]Peptide	[b]Θ_{222}	[c]Measured f_α	[d]Computed f_α	ΔG_{AC} (kcal mol^{-1})	[e]$\Delta G_{per\ residue}$ (kcal mol^{-1})
$A_8Q_3L_4$-0.55	–4400	0.092	0.13	1.32±0.06	0.84±0.02
$A_8Q_3L_4$-2.00	–6500	0.158	0.14	1.06±0.04	0.39±0.01
$A_8Q_3L_4$-2.86	–8200	0.212	0.21	0.82±0.04	0.23±0.01
$A_8Q_3L_4$-4.72	–10700	0.292	0.33	0.56±0.04	0.11±0.01
$A_8Q_3L_4$-5.51	–13500	0.380	0.35	0.27±0.04	0.042±0.006
$A_8Q_3L_4$-5.54	–14000	0.396	0.33	0.27±0.04	0.040±0.006
melittin	–3000	0.060	0.013	1.62±0.06	1.04±0.04
TMX-3	-6500	0.220	0.031	0.74±0.03	0.11±0.01

[a]See Table 1

[b]molar ellipticity, deg cm^2 dmol^{-1}

[c]fractional helicity in buffer. See Methods.

[d]fractional helicity, determined using the computer program AGADIR[6-9]. The estimated uncertainty in these values is estimated by the authors of AGADIR to be 6%.

[e]These are per-residue free energies computed using $\Delta G_{per\ residue} = \Delta G_{AC}/f_\alpha n$, where f_α is the fractional helicity and n is the number of residues in the sequence.

Determination of the partitioning free energies into membranes of partially-folded peptides

The free energies of peptide partitioning into LUV formed from POPC and POPC/POPG (1:1) were determined by both CD and fluorescence spectroscopy titration following the procedures of White *et al.*[10] (see Methods). The results are summarized in **Table S2**, below.

Typical titration data obtained by CD spectroscopy in **Fig. S3** for $A_8L_4Q_3$–5.54, where the upper panels (**A and B**) correspond to a titration with POPC membranes and the lower panels (**C and D**) to titrations with POPC/POPG membranes. The helicity of $A_8L_4Q_3$–5.54 increases steadily with additions of lipid (**panels A and C**). Molar ellipticity at 222 nm was used to determine quantitatively partitioning isotherms[10-12]. These were fit by least-squares minimization to obtain the maximum ellipticity ($[\Theta]_{max}$), and mole-fraction partition coefficients (K_x) and consequently ΔG_{CD} (**panels B and D**), as described in Methods.

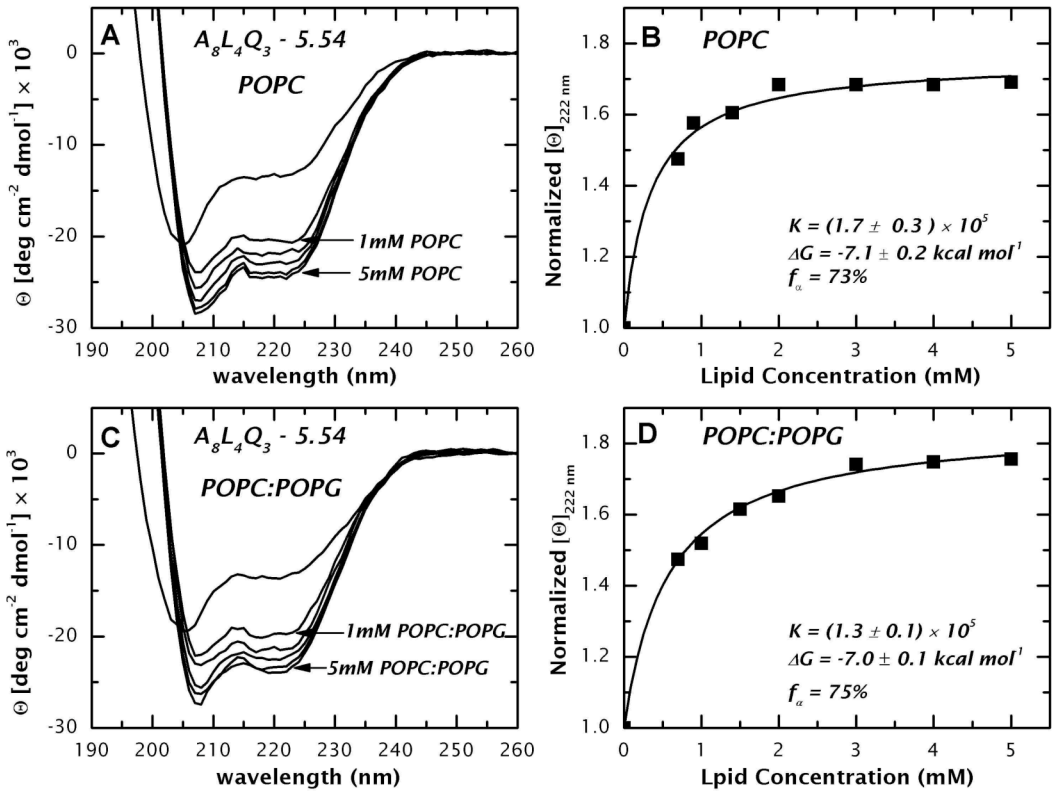

Figure S3. Typical titration data obtained by CD spectroscopy for $A_8L_4Q_3$–5.54, where the upper panels (**A and B**) correspond to a titration with POPC membranes and the lower panels (**C and D**) to titrations with POPC/POPG membranes

Because Trp fluorescence is sensitive to its dielectric environment, membrane association of peptides results in a blue-shift in the Trp fluorescence wavelength

maximum (λ_{max}) that can be used to measure membrane partitioning[10] by titration of peptide solutions with LUV, as discussed in detail by Ladokhin *et al.*[13]. Typical fluorescence spectra and titration data are presented for $A_8L_4Q_3$–5.54 in **Fig. S4**. All spectra were corrected for scattering artifacts[13]. For both POPC and POPC/POPG (**panels A and C**, respectively), λ_{max} = 350 nm in buffer, shifting to about 335 nm in the presence of lipid. Titration curves obtained from the change in fluorescence at 325 nm are shown in panels B and D for POPC and POPC/POPG, respectively. In a manner similar to that for the CD titrations, the data shown in **Fig. S4** were fit by least-squares minimization to obtain the mole-fraction partition coefficient K_X and the maximum intensity increase I_∞ (**panels B and D**), as described in Methods.

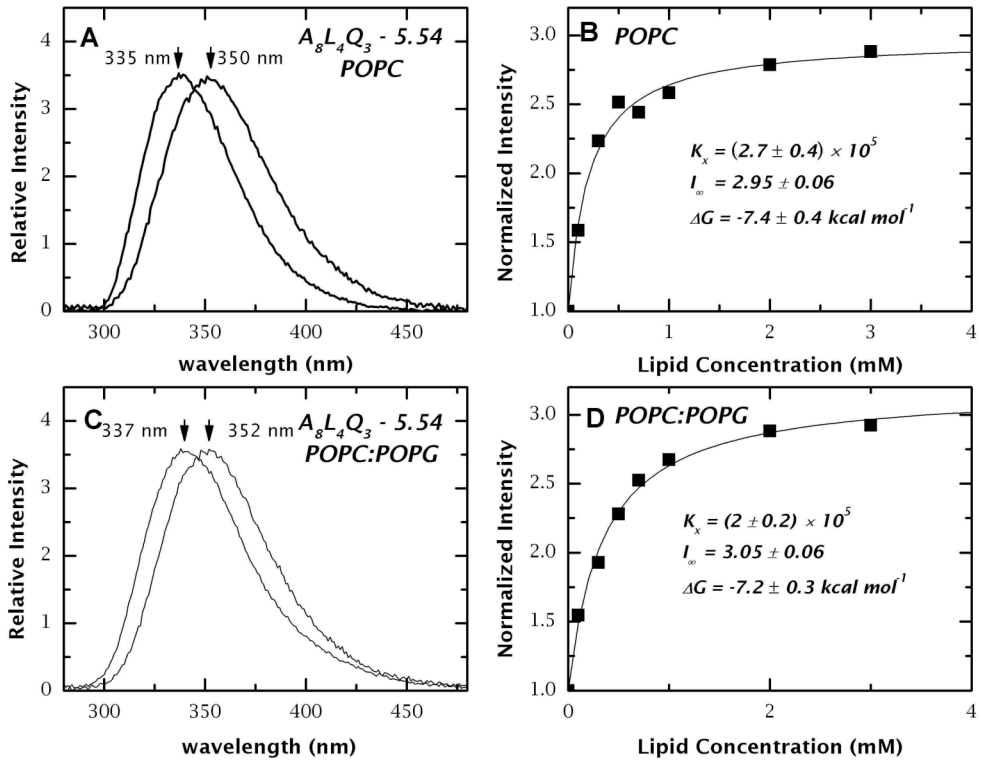

Figure S4. Typical fluorescence spectra and titration data for $A_8L_4Q_3$–5.54. All spectra were corrected for scattering artifacts[13]. For both POPC and POPC/POPG (**panels A and C**, respectively), λ_{max} = 350 nm in buffer, shifting to about 335 nm in the presence of lipid. Binding curves determined from data such as those of panels A and C (see Methods) are shown in **panels B and D** for POPC and POPC:POPG, respectively.

Table S2. Free energies of transfer ΔG_{CD} of the $A_8Q_3L_4$ family of peptides from buffer into neutral and anionic large unilamellar vesicles.

[a]Peptide	[b]Θ_{222}	[c]f_α	[d]ΔG_{CD} POPC	[d]ΔG_{CD} [e]POPC:POPG	[d]ΔG_{CD} POPC	[d]ΔG_{CD} [e]POPC:POPG
			[f]*Fluorescence Titration*		[f]*CD Titration*	
$A_8Q_3L_4$-0.55	–12000	0.30	–5.2±0.3	–5.0±0.5	—	—
$A_8Q_3L_4$-2.00	–13000	0.36	–5.7±0.2	–5.4±0.2	—	—
$A_8Q_3L_4$-2.86	–16000	0.46	–5.9±0.2	–5.8±0.2	—	—
$A_8Q_3L_4$-4.72	–22000	0.67	–6.4±0.2	–6.7±0.1	–6.1±0.1	–6.1±0.1
$A_8Q_3L_4$-5.51	–24500	0.72	–7.3±0.1	–7.0±0.1	–7.3±0.2	–7.1±0.2
$A_8Q_3L_4$-5.54	–25000	0.73	–7.4±0.2	–7.2±0.1	–7.0±0.2	–7.1±0.2

[a]see Table 1

[b]maximum molar ellipticity [Θ_{max}] deg $cm^2 dmol^{-1}$ (see Fig. S3).

[c]fractional helicity obtained by CD experiments (see Methods). When CD experiments were not possible, approximations were made from ellipticities of titrations of the peptides with methanol.

[d]kcal mol^{-1}

[e]POPC:POPG = 1:1

[f]see Methods

Peptide folding in the membrane interface: ΔG_{BD}

The free energies of folding of the peptides in the interface are obtained by simple summation of the other legs of the thermodynamic cycle shown in **Fig. 1a**. The results are summarize in **Table S3**, below.

Table S3. Free energy of folding ΔG_{BD} in the POPC bilayer interface.

[a]Peptide	[b]ΔG_{BD} kcal mol^{-1}	[c]$\Delta G_{residue}$ kcal mol^{-1}
$A_8Q_3L_4$-0.55	–0.35±0.30	–0.07±0.06
$A_8Q_3L_4$-2.00	–1.11±0.20	–0.18±0.03
$A_8Q_3L_4$-2.86	–1.55±0.20	–0.20±0.02
$A_8Q_3L_4$-4.72	–2.51±0.20	–0.23±0.02
$A_8Q_3L_4$-5.51	–3.50±0.11	–0.28±0.01
$A_8Q_3L_4$-5.54	–3.60±0.11	–0.29±0.02
Melittin-5.16	–5.31±0.21	–0.27±0.01
TMX3-3.32 pH=7.6	-7.21±0.20	-0.24±0.02

[a]Table 1

[b]Computed from the thermodynamic cycle of **Fig. 1a** and the data of **Tables 1**, **S1**, and **S2**.

[c]These are per-residue free energies computed using $\Delta G_{residue} = \Delta G_{AC}/f_{\alpha}n$, where f_{α} is the fractional helicity (**Table S2**) and n is the number of residues in the sequence.

Leu-Leu interactions and helix stability

Table S4. Sequences of peptides used by Luo and Baldwin[14] for studying the role of Leu-Leu interactions in helix stability. Even in this case, the helicity in water increases with the hydrophobic moment (**Fig. S5**).

Sequence	Sidechain interaction	[a]f_α	[b]μH
Ac-KAAAAKAALAKLAAAKGY-NH_2	(i,i+3)	26%	0.57
Ac-KAAAAKAALAKALAAKGY-NH_2	(i,i+4)	37%	1.33
Ac-ELAALKAKLAALKAKAGY-NH_2	2(i,i+3) + (i,i+4)	46%	1.48
Ac-ELAALKAKLAALKAKLGY-NH_2	2(i,i+3) + 2(i,i+4)	53%	1.62

[a] Percentage values given by Luo and Baldwin[14] of helical content in buffer.

[b] Values of hydrophobic moment computed by MPEx.

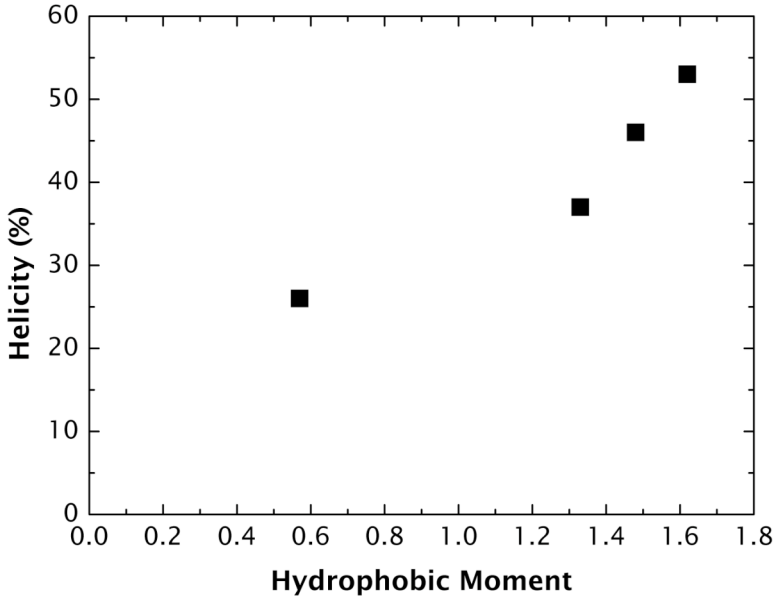

Figure S5. Plot the helicities in **Table S4** versus hydrophobic moment.

Consistency of the measured values of helix content with values calculated using AGADIR

As shown below in **Fig. S6**, the relation between the measured values of helicity and values computed with AGADIR[6-9] is described well by a straight line forced through the origin (y = ax). The slope of the curve is 1.03(±0.03). This means that AGADIR is useful for estimating helicities in water. For instance, the peptide $A_8L_4Q_3$-0.55 is 9% helical in water, while AGADIR predicts 12.8 % ± 6%.

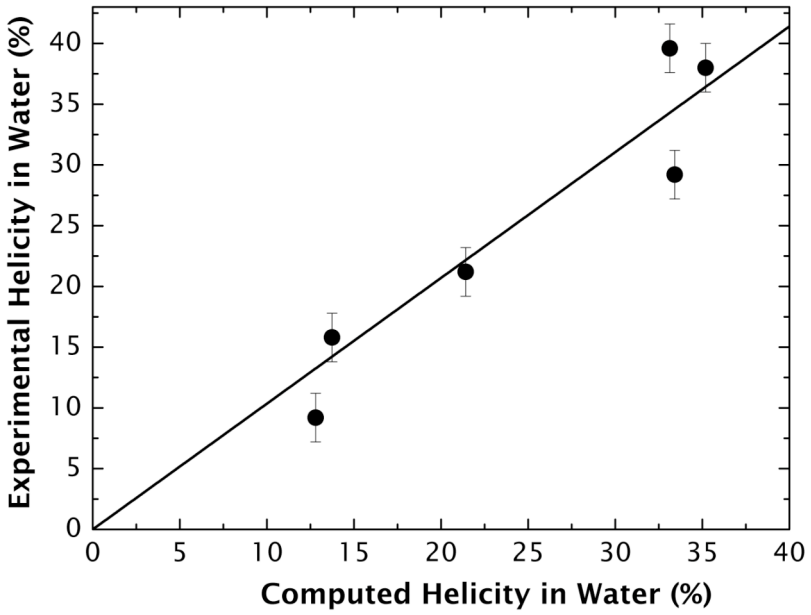

Figure S6. Experimentally determined helical content for the $A_8L_4Q_3$ peptide family in water plotted against helical contents computed using the program Agadir[6-9].

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