SUPPORTING INFORMATION

Peptides at the interface: self-assembly of amphiphilic designer peptides and their membrane interaction propensity

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Supporting References
High-performance liquid chromatography (HPCL) and mass spectrometry (MS) analysis of A₆YD, V₄WD₂ and A₆YK

The peptides ac-A₆YD (A₆YD) and ac-V₄WD₂ (V₄WD₂) were custom synthesized and purified by Peptide 2.0 (Chantilly, VA, USA), ac-A₆YK-NH₂ (A₆YK) was derived from piCHEM GmbH (Graz, Austria).

**HPLC Data**

| Parameter          | Value                                          |
|--------------------|------------------------------------------------|
| HPLC Column        | Merck (250x4.6mm I.D.) C18                    |
| Detection wavelength | 220 nm                                      |
| Gradient           | 10-30% B in 20 min                            |
| Buffer A           | 0.05% TFA in H₂O                               |
| Buffer B           | 0.05% TFA in 90% CH₃CN                        |
| Gradient A         | A                                             |
| 0.01 min A         | 90%                                           |
| 20.0 min A         | 70%                                           |
| 20.1 min A         | 0%                                            |
| 30.0 min A         | STOP                                          |
| Gradient B         | B                                             |
| 0.01 min B         | 10%                                           |
| 20.0 min B         | 30%                                           |
| 20.1 min B         | 100%                                          |
| 30.0 min B         | STOP                                          |

**Figure S1.** HPLC analysis of A₆YD
Figure S2. MS analysis of A₆ YD
**HPLC Data**

- **Column**: 4.6×250mm, Venusil MP C18-5
- **Solvent A**: 0.1% trifluoroacetic in 100% acetonitrile
- **Solvent B**: 0.1% trifluoroacetic in 100% water

**Figure S3.** HPLC analysis of V₄WD₂

| Peak No. | Ret Time (min) | Height (mV) | Area (cm²) | Conc. (ppm) |
|----------|----------------|-------------|------------|-------------|
| 1        | 12.463         | 1059.054    | 9325.810   | 0.3027      |
| 2        | 13.587         | 3466.117    | 32420.588  | 1.0522      |
| 3        | 13.938         | 544.651     | 2701.000   | 0.0877      |
| 4        | 14.357         | 3632.474    | 28619.693  | 0.9289      |
| 5        | 14.767         | 1170.771    | 12365.510  | 0.4013      |
| 6        | 15.180         | 259670.766  | 2937175.250| 95.3268     |
| 7        | 15.870         | 791.827     | 9079.810   | 0.2947      |
| 8        | 19.238         | 5359.218    | 49477.816  | 1.6058      |

**Total** 100.0000
Figure S4. MS analysis of $V_4WD_2$
Sequence: Acetyl-A-A-A-A-A-A-Y-K-NH2 Acetat
BatchNr.: X25-110701 MW: 776.9 av.

acquired by: MHä

Method: C:\CHEM32\l\ --> 
H2O (0,1% TFA) / CH3CN (0,1% TFA)
05% B auf 50% B in 40min
Nucleosil C18; 150 x 4 mm; 5 µm; 1 ml/min

Figure S5. HPLC analysis of A₆YK
Figure S6. MS analysis of $A_6 YK$
Structure formation of V₄WD₂ and A₆YD explored by SAXS and ATR-FTIR

Figure S7. SAXS and ATR-FTIR measurements of different A₆YD and V₄WD₂ concentrations. The peptide V₄WD₂ does not show ordered supramolecular structures for a large concentration regime. SAXS is extremely sensitive to aggregates, and an indicative rise in intensity at low q-values can already be seen at very low peptide concentrations (A). This suggests that self-assembly starts slowly and continuously and a distinct CAC value, such as it is conventionally determined (e.g. with fluorescence techniques), is probably not applicable. Although we have not observed ordered structures with SAXS, ATR-FTIR measurements suggest that the aggregates show internal β-sheet characteristics, seen as an amide I peak at 1630 cm⁻¹ (B). Most probably the electrostatic repulsion between charged aspartic acid residues is high and thus prevents the formation of large supramolecular structures on a long range order. Nevertheless, valine has a high propensity for hydrogen bonding and might to a certain extent outbalance electrostatic repulsion. Thus, assembly into small aggregates, held together by weak intermolecular hydrogen bonds is likely. For A₆YD, scattering patterns suggest that self-assembly into highly ordered structures starts between 1-5 mM, with characteristics of a cylindrical architecture. At very high A₆YD concentrations (> 30 mM) the SAXS patterns show the additional contribution of a structure factor at low q-values (q<0.4 nm⁻¹). This means that the individual superstructures are not in a dilute state any more, but distances of the cylinders relative to each other come into the same order of magnitude as the distances inside the individual cylinders. The distinct shape of this peak suggests that the cylinders are not only
densely packed, but probably also arranged in an aligned orientation (C). ATR-FTIR measurements display a peak at 1636 cm$^{-1}$ in the amide I region, which can be attributed to β-type structures. Like for V$_4$WD$_2$, we observe a concentration-dependent scaling of the IR signal (D).

In conclusion, a combination of both techniques - SAXS and ATR-FTIR - shows that the morphology of the assemblies is rather sequence-dependent and does not change significantly with concentration. It means that the individual structural characteristics are developed already at low peptide concentrations, but are getting more pronounced the higher the concentration.

**Table S1.** SAXS analysis of 11 mM A$_6$YD

| Fitting Parameters                        |       |
|-------------------------------------------|-------|
| Incoherent background scattering          | 0.06  |
| Extrapolated intensity at zero angle ($I_0$)| 3.9   |
| Outer cylinder radius in nm ($a_1$)       | 3.3   |
| Inner cylinder radius in nm ($a_2$)       | 1.6   |
| Ellipticity factor                        | 0.83  |
| Electron density ratio ($\rho_2/\rho_1$)  | 1.04  |
| Porod constant                            | 0.05  |
| Porod exponent                            | 0.79  |
| Hard sphere radius in nm ($R_s$)          | 2.6   |
| Volume fraction ($\phi$)                  | 0.20  |
| Interparticle potential, width of the square-well ($\lambda$) | 1.9   |
| Interparticle potential, depth of the square-well ($\epsilon$) | -0.07 |

**Table S1** summarizes the parameters obtained by fitting the scattering pattern of 11 mM A$_6$YD with a cylinder-shell model, combined with a sticky hard sphere interaction term. This structure factor was used for simplicity. The structures show an outer radius of 3.3 nm, and an inner cylinder radius of 1.6 nm, resulting in a shell thickness of ~1.7 nm. The high volume fraction - in combination with the derived values for the interparticle potential - indicates an attractive interaction amongst the self-assembled structures. A hard sphere radius of 2.6 nm seems in contradiction to an outer cylinder radius of 3.3 nm. However, as the cylinders display either slight cross-sectional ellipticity or a slight distribution in size, both values are in agreement. Consequently, the cylindrical structures are tightly packed.
EPR spectroscopy of DPPC in the absence and presence of A₆YD and V₄WD₂, at different peptide concentrations, different temperatures (25 °C/50 °C), different spin labels (5-DSA/7-MeDSA)

**Figure S8.** EPR spectra of DPPC in the presence of the amphiphilic designer peptides A₆YD and V₄WD₂ at 25 °C (lamellar gel (Lβ') phase) and 50 °C (liquid crystalline (Lα) phase), with two different spin labels used (5-DSA and 7-MeDSA). The spectra are shifted in the vertical axis for clearer visibility.
Table S2. Derived EPR order parameters ($S$) for DPPC + peptides

The order parameters ($S$) were obtained as described in the main article (see section Electron paramagnetic resonance (EPR) spectroscopy on DPPC MLVs).

| order parameter ($S$) | 25 °C, 5-DSA | 50 °C, 5-DSA | 25 °C, 7-MeDSA | 50 °C, 7-MeDSA |
|-----------------------|---------------|---------------|----------------|----------------|
| pure DPPC             | 0.99          | 0.95          | 0.72           | 0.69           |
| DPPC:A$_6$YD=50:1     | 0.97          | 0.96          | 0.69           | 0.70           |
| DPPC:A$_6$YD=25:1     | 0.97          | 0.97          | 0.72           | 0.70           |
| DPPC:A$_6$YD=10:1     | 0.98          | 0.96          | 0.72           | 0.70           |
| DPPC:A$_6$YD=5:1      | 0.97          | 0.95          | 0.73           | 0.70           |
| DPPC:V$_4$WD$_2$=50:1 | 0.95          | 0.98          | 0.73           | 0.73           |
| DPPC:V$_4$WD$_2$=25:1 | 0.83          | 0.81          | 0.71           | 0.68           |
| DPPC:V$_4$WD$_2$=10:1 | 0.75          | 0.77          | 0.69           | 0.68           |
| DPPC:V$_4$WD$_2$=5:1  | 0.75          | 0.76          | 0.74           | 0.74           |

Table S3. Derived EPR isotropic splitting constants ($a'$) for DPPC + peptides

The isotropic splitting constants ($a'$) were obtained as described in the main article (see section Electron paramagnetic resonance (EPR) spectroscopy on DPPC MLVs).

| isotropic splitting constant ($a'$) | 25 °C, 5-DSA | 50 °C, 5-DSA | 25 °C, 7-MeDSA | 50 °C, 7-MeDSA |
|------------------------------------|---------------|---------------|----------------|----------------|
| pure DPPC                          | 14.4          | 14.6          | 15.6           | 15.5           |
| DPPC:A$_6$YD=50:1                  | 14.5          | 14.7          | 16.1           | 15.5           |
| DPPC:A$_6$YD=25:1                  | 14.5          | 14.4          | 15.6           | 15.5           |
| DPPC:A$_6$YD=10:1                  | 14.4          | 14.5          | 15.6           | 15.6           |
| DPPC:A$_6$YD=5:1                   | 14.5          | 14.5          | 15.7           | 15.6           |
| DPPC:V$_4$WD$_2$=50:1              | 14.7          | 14.3          | 15.5           | 15.1           |
| DPPC:V$_4$WD$_2$=25:1              | 15.3          | 15.0          | 15.3           | 15.3           |
| DPPC:V$_4$WD$_2$=10:1              | 15.2          | 15.1          | 15.0           | 15.0           |
| DPPC:V$_4$WD$_2$=5:1               | 15.0          | 15.0          | 15.7           | 15.8           |
**Negative-staining Transmission Electron Microscopy micrographs compared to Cryogenic Transmission Electron Microscopy (Cryo-TEM) micrographs**

The procedure of negative-staining is often subject to a critical survey, since the sample solution is blotted several times and left to dry on the grid. Especially for peptide samples, where supramolecular structure development is often concentration-dependent, evaporation and blotting can lead to the formation of structures that are not present in the original solution. Cryo-TEM is known to be less invasive and should preserve vitrified structures. We were interested in differences and similarities, and investigated the peptide $\text{A}_6\text{YK}$ with both techniques.

Negative-staining TEM sample preparation:

Samples were adsorbed onto a glow-discharged carbon-coated copper grid and allowed to settle for 60 seconds. Excess fluid was carefully removed with filter paper and immediately replaced by 5 µl of a 2 % (w/v) uranyl acetate staining solution. It was allowed to settle for 30 seconds, blotted and replaced by another 5 µl of fresh solution. After 30 seconds the staining solution was removed and the sample was air-dried. Imaging was done by using a Fei Tecnai G² 20 transmission electron microscope (Eindhoven, The Netherlands) operating at an acceleration voltage of 120 kV.

Cryo-TEM sample preparation:

Sample solution was applied to a glow-discharged carbon-coated copper grid, blotted to create a thin film, plunged into liquid ethane and transferred to liquid nitrogen. Vitrified specimens were transferred onto a Gatan 626-DH cryo transfer specimen holder. Imaging was done at cryogenic temperatures in a Tecnai T12 (FEI, The Netherlands) microscope, operated at 120 kV.

Figure S11 shows a direct comparison of a negatively-stained (C) and a vitrified $\text{A}_6\text{YK}$ sample measured under cryogenic conditions (D). Negatively-stained samples exhibit a slightly denser network of fibers (C), which possibly originates from the drying procedure. In contrast, cryo-TEM samples seem to show a more aligned orientation of fibers (D). Nevertheless, both examples display the same structural morphology of $\text{A}_6\text{YK}$ assemblies, which are characterized by almost the same sizes: lengths extend to several hundred of nanometers, whereas the fibers show diameters of 4-10 nm with negative-staining TEM, and 8-12 nm with cryo-TEM. Thus, we conclude that the micrographs of both techniques are of similar quality and yield the same information content.
Self-assembly and membrane interaction propensity of $A_6YK$

In order to test a positively charged analog of $A_6YD$, the peptide $A_6YK$ was designed and compared to $A_6YD$ and $V_4WD_2$ regarding self-assembling behavior and membrane activity. Figure S9 shows the amino acid sequence of the peptide. The critical aggregation concentration (CAC) was determined to be in the range of 1 mM (Fig. S10). Above this concentration the peptide self-assembles into cylindrical structures, which show an antiparallel internal organization of monomers, most probably stabilized by hydrogen bonding interactions. The supramolecular cylinders are several hundred nanometers in length and ~4-12 nm in diameter (Fig. S11).

![Amino acid sequence of the peptide $A_6YK$. The hydrophobic part (green), as well as the positively charged domain (orange) are highlighted.](image)

**Figure S9.** Amino acid sequence of the peptide $A_6YK$. The hydrophobic part (green), as well as the positively charged domain (orange) are highlighted.

![Determination of the critical aggregation concentration by a DPH fluorescence assay. $A_6YK$ shows a CAC of ~1 mM.](image)

**Figure S10.** Determination of the critical aggregation concentration by a DPH fluorescence assay. $A_6YK$ shows a CAC of ~1 mM.
Figure S11. Supramolecular structure formation of 11 mM A₆YK. The SAXS scattering pattern of A₆YK shows typical characteristics of a cylindrical morphology (A). With ATR-FTIR (B) we observed a major amide I peak at 1626 cm⁻¹, characteristic for either an asymmetric in plane bending vibration of lysine’s NH₃⁺ group or intermolecular β-sheet interactions, most probably a combination of both. A minor peak was found at 1670 cm⁻¹, which we assigned to the antiparallel component of β-type structures. We assume that peptide monomers as the building blocks of the supramolecular cylinders are aligned in an antiparallel way. The presence of cylindrical structures was confirmed with TEM (C and D). Negative-staining TEM (C) shows a network of long fibers, characterized by diameters between 4-10 nm and lengths extending several micrometers. The same structures were also observed with cryo-TEM, although their diameters appeared slightly larger (8-12 nm).
A₆YK’s propensity to interact with synthetic as well as biological membranes was assessed by a variety of biophysical techniques, namely differential scanning calorimetry (DSC), electron paramagnetic resonance spectroscopy (EPR), and small angle X-ray scattering (SAXS). Samples containing different lipid:peptide ratios (5:1, 10:1, 25:1, 50:1 mol/mol, orange curves) were prepared as described in the main article and compared to controls without peptide (grey curves). Single component phospholipid bilayers (Dipalmitoyl phosphatidylcholine multilamellar vesicles, DPPC MLVs) and low density lipoprotein (LDL) served as model systems. As can be seen in Figure S12A, B, and C the peptide A₆YK has almost no influence on DPPC MLVs. The thermotropic phase behavior of DPPC remained unaffected by the presence of A₆YK and displayed the well-characterized pre- and main-transition at 36 °C and 41.7 °C, respectively (Fig. S12A and Table S2). EPR spectra at 25 °C with a 5-DSA spin label showed typical characteristics of anisotropic lipid motion in membranes and did not differ whether peptides were present or not. This means that A₆YK did not affect the mobility of the spin label in both systems, DPPC and LDL (Fig. S12B and D). SAXS patterns of pure DPPC at 25 °C showed three reflection orders with a characteristic d-spacing of 6.41 nm, which was maintained in the presence of A₆YK (Fig. S12C). Also LDL particles displayed the same characteristics of the scattering pattern as controls when A₆YK was added. The only difference could be observed in the low q-range where we noticed a slight increase in intensity. Since A₆YK has a very low CAC and all peptide concentrations are applied above the CAC we expect also a contribution of the supramolecular peptide assemblies in this particular region.

Figure S12. Biophysical characterization of DPPC-A₆YK interactions by DSC (A), EPR spectroscopy (B), SAXS (C) and LDL-A₆YK interactions by EPR spectroscopy (D) and SAXS (E).
**Table S4.** DSC parameters of pure DPPC and DPPC-A₆YK-mixtures of varying lipid:peptide molar ratios

| Sample                | T$_{pre}$ (°C) | T$_m$ (°C) | ΔH (kcal/mol/°C) | ΔT$_{1/2}$ |
|-----------------------|----------------|------------|------------------|------------|
| DPPC                  | 36.0           | 41.7       | 9.8              | 0.12       |
| DPPC + A₆YK 50:1      | 35.8           | 41.7       | 9.2              | 0.13       |
| DPPC + A₆YK 25:1      | 35.6           | 41.7       | 9.6              | 0.12       |
| DPPC + A₆YK 10:1      | 35.6           | 41.7       | 8.7              | 0.15       |
| DPPC + A₆YK 5:1       | 35.2           | 41.7       | 9.0              | 0.23       |
EPR spectroscopy of LDL in the absence and presence of $A_6YD$ and $V_4WD_2$, at different peptide concentrations, different temperatures (10°C/25°C/37°C), different spin labels (5-DSA/7-MeDSA)

**Figure S13.** EPR spectra of LDL in the presence of the amphiphilic designer peptides $A_6YD$ and $V_4WD_2$ at 10 °C (below the core lipid transition temperature), 25 °C (around the core lipid transition temperature) and 37 °C (above the core lipid transition temperature), with two different spin labels used (5-DSA, which probes lipid mobility in the surface lipid monolayer and 7-MeDSA, which probes lipid mobility in the hydrophobic core region). The spectra are shifted in the vertical axis for clearer visibility.
Table S5. Derived EPR order parameters (\(S\)) and rotational correlation times (\(\tau_c\)) for LDL + peptides

The order parameters (\(S\)) were obtained as described in the main article. The EPR spectra of the 7-MeDSA spin label, which probes mobility in the core region, change from anisotropic motion to isotropic motion at temperatures above the core melting transition. As a result, the hyperfine tensors \(T_{\parallel}'\) and \(T_{\perp}'\) could not be determined without ambiguity. Therefore, the rotational correlation time (\(\tau_c\)) was used as a means to describe spin label mobility and was calculated as described in the main article.

|                   | \(5\)-DSA, 10 °C | \(5\)-DSA, 25 °C | \(5\)-DSA, 37 °C | 7-MeDSA, 10 °C | 7-MeDSA, 25 °C | 7-MeDSA, 37 °C | \(7\)-MeDSA, 37 °C | \(\tau_c\) (ns) |
|-------------------|------------------|------------------|------------------|----------------|----------------|----------------|------------------|-----------------|
| native LDL        | 0.82             | 0.73             | 0.69             | 0.73           | 0.65           | n.d.           | 4.20             |
| LDL:A6YD =50:1    | 0.82             | 0.74             | 0.67             | 0.78           | 0.63           | n.d.           | 4.38             |
| LDL:A6YD =25:1    | 0.81             | 0.73             | 0.68             | 0.78           | 0.63           | n.d.           | 4.19             |
| LDL:A6YD =10:1    | 0.82             | 0.74             | 0.69             | 0.77           | 0.63           | n.d.           | 4.03             |
| LDL:V4WD2 =50:1   | 0.85             | 0.75             | 0.68             | 0.80           | 0.63           | n.d.           | 4.06             |
| LDL:V4WD2 =25:1   | 0.85             | 0.74             | 0.68             | 0.80           | 0.63           | n.d.           | 4.18             |
| LDL:V4WD2 =10:1   | 0.85             | 0.76             | 0.69             | 0.80           | 0.63           | n.d.           | 4.20             |

Table S6. Derived EPR isotropic splitting constants (\(a'\)) for LDL + peptides

The isotropic splitting constants (\(a'\)) were obtained as described in the main article.

| isotropic splitting constant (\(a'\)) | \(5\)-DSA, 10 °C | \(5\)-DSA, 25 °C | \(5\)-DSA, 37 °C | 7-MeDSA, 10 °C | 7-MeDSA, 25 °C | 7-MeDSA, 37 °C |
|-------------------------------------|------------------|------------------|------------------|----------------|----------------|----------------|
| native LDL                          | 15.6             | 15.1             | 15.0             | 15.7           | 15.1           | n.d.           |
| LDL:A6YD =50:1                      | 15.6             | 15.2             | 15.0             | 15.3           | 15.0           | n.d.           |
| LDL:A6YD =25:1                      | 15.8             | 15.1             | 15.0             | 15.3           | 15.0           | n.d.           |
| LDL:A6YD =10:1                      | 15.6             | 15.1             | 14.9             | 15.4           | 15.0           | n.d.           |
| LDL:V4WD2 =50:1                     | 15.2             | 15.2             | 15.0             | 14.9           | 15.1           | n.d.           |
| LDL:V4WD2 =25:1                     | 15.2             | 15.1             | 14.9             | 14.9           | 15.1           | n.d.           |
| LDL:V4WD2 =10:1                     | 15.2             | 15.1             | 15.0             | 14.9           | 15.0           | n.d.           |
SAXS of LDL in the absence and presence of peptides \( A_6 Y D \) and \( V_4 W D_2 \)

**Figure S14.** SAXS patterns of LDL in the presence of \( A_6 Y D \) (A) and \( V_4 W D_2 \) (B) only indicate a slight shift of the first order maximum at the highest peptide concentration. All spectra were shifted in the y-axes for clearer visibility. The interaction of \( A_6 Y K \) with LDL is shown in Fig. S12E.

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