



Supporting Information

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Helical arrangement of interstrand stacked pyrenes in a DNA framework.

Vladimir Malinovskii, Florent Samain and Robert Häner

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1) Experimental Part

The required pyrene building block was synthesized according to a published procedure (S. M. Langenegger, R. Häner, *Chem. Commun.* **2004**, 2792-2793.) Nucleoside phosphoramidites from *Transgenomic* (Glasgow, UK) were used for oligonucleotide synthesis. Oligonucleotides **1-10** were prepared *via* automated oligonucleotide synthesis by a standard synthetic procedure ('trityl-off' mode) on a 394-DNA/RNA synthesizer (*Applied Biosystems*). Cleavage from the solid support and final deprotection was done by treatment with 30% NH_4OH solution at 55°C overnight. All oligonucleotides were purified by reverse phase HPLC (LiChrospher 100 *RP-18*, 5 μm , Merck), *Bio-Tek Instruments Autosampler 560*); eluent A = $(\text{Et}_3\text{NH})\text{OAc}$ (0.1 M, pH 7.4); eluent B = MeCN; elution at 40°C; gradient 5 – 20% B over 30 min. Mass spectrometry of oligonucleotides were performed with a Sciex QSTAR pulsar (hybrid quadrupole time-of-flight, ESI negative mode) mass spectrometer, *Applied Biosystems*.

All the measurements were performed in potassium phosphate buffer (10 mM, 100 mM NaCl, pH 7.0) for 1.0 μM oligonucleotide concentration (1.0 μM of each strand in case of duplex), $\epsilon_{260} = 9000$ was used for pyrene units. Thermal denaturation experiments were carried out on *Varian Cary-100 Bio-UV/VIS* spectrophotometer equipped with a *Varian Cary-block* temperature controller and data were collected with *Varian WinUV* software at 245, 260 and 354 nm (cooling-heating-cooling cycles in the temperature range of 20-90°C, temperature gradient of 0.5°C/min). Temperature melting (T_m) values were determined as the maximum of the first derivative of the smoothed melting curve.

Temperature dependent UV-VIS spectra were collected with an optic path of 1 cm over the range of 210-500 nm at 10-90 °C with a 10 °C interval on *Varian Cary-100 Bio-UV/VIS* spectrophotometer equipped with a *Varian Cary-block* temperature controller. The cell compartment was flushed with N_2 .

Temperature dependent fluorescence data were collected on a *Varian Cary Eclipse* fluorescence spectrophotometer equipped with a *Varian Cary-block* temperature controller (excitation at 354 nm; excitation and emission slit width of 5 nm) using 1 per 1 cm quartz cuvettes. *Varian Eclipse* software was used to investigate the fluorescence of the different pyrene-containing oligonucleotides at a wavelength range of 375-700 nm in the temperature range of 10-90 °C.

CD spectra were recorded on a *JASCO J-715* spectrophotometer using quartz cuvettes with an optic path of 1 cm.

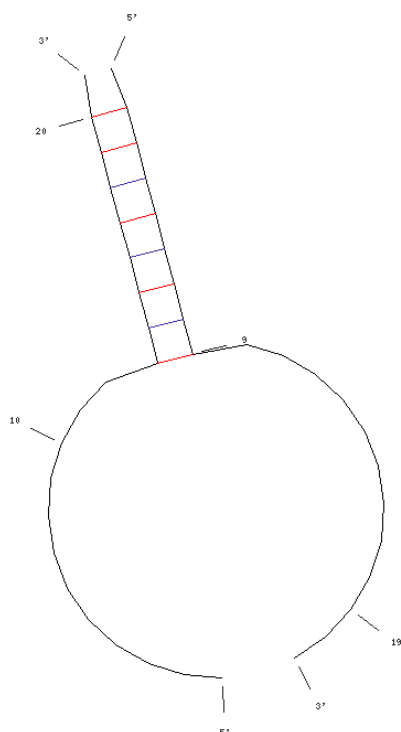
2. Example of calculated T_m of modified duplex

Scheme 1. Calculation of melting temperature for duplex **9*10**, excluding interstrand interaction within the modified part.

$$\Delta G = -7.9 \text{ kcal/mol} \quad \Delta H = -69.3 \text{ kcal/mol} \quad \Delta S = -197.9 \text{ e.u.} \quad T_m = 30.7^\circ\text{C}$$

Structural element	$\delta\delta G$	Information
External loop	+0.00	24 ss bases & 1 closing helices
Stack	-1.04	External closing pair is G ¹³ -C ⁹
Stack	-1.02	External closing pair is A ¹⁴ -T ⁸
Stack	-1.04	External closing pair is G ¹⁵ -C ⁷
Stack	-1.02	External closing pair is A ¹⁶ -T ⁶
Stack	-1.18	External closing pair is G ¹⁷ -C ⁵
Stack	-1.19	External closing pair is T ¹⁸ -A ⁴
Stack	-1.98	External closing pair is G ¹⁹ -C ³
Helix	-8.45	8 base pairs
External loop	+0.53	Closing pair is C ²⁰ -G ²

Output of sir_graph
by D. Stewart and M. Zuker



$$\Delta G = -7.926 \text{ kcal/mol} \quad \Delta H = -69.3 \text{ kcal/mol}$$

See: the DINAMelt server <http://www.bioinfo.rpi.edu/applications/hybrid/twostate.php>

Prediction of melting profiles for nucleic acids. Markham, N. R. & Zuker, M. (2005) DINAMelt web server for nucleic acid melting prediction. *Nucleic Acids Res.*, **33**, W577-W581.

3) Spectroscopic Data.

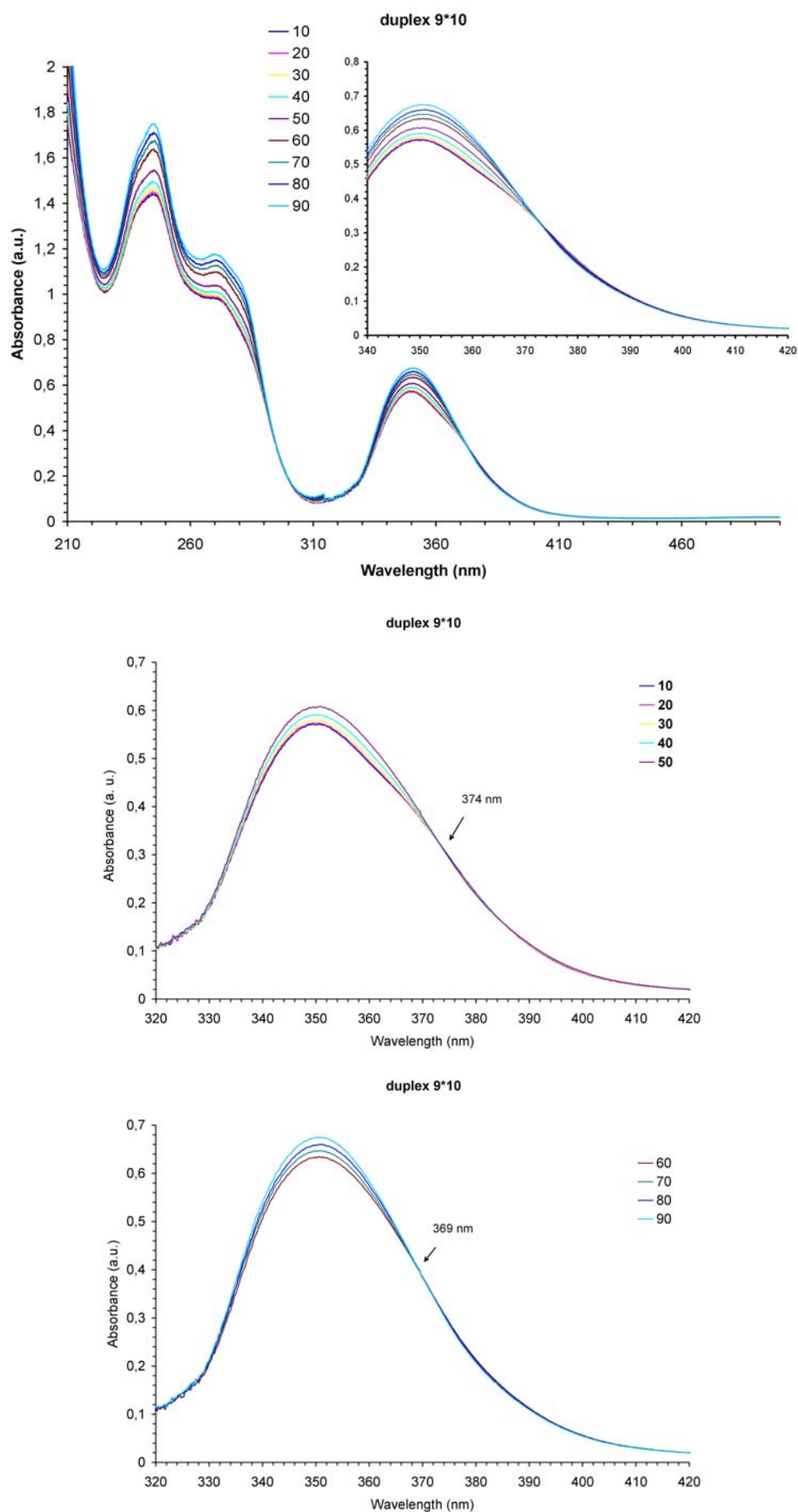


Figure 1. Temperature dependent UV-Vis of a) duplex **9*10** (210-500 nm, 10-90 °C); b) isosbestic point at 374 nm (320-420 nm, 10-40 °C); c) isosbestic point at 369 nm (320-420 nm, 60-90 °C).

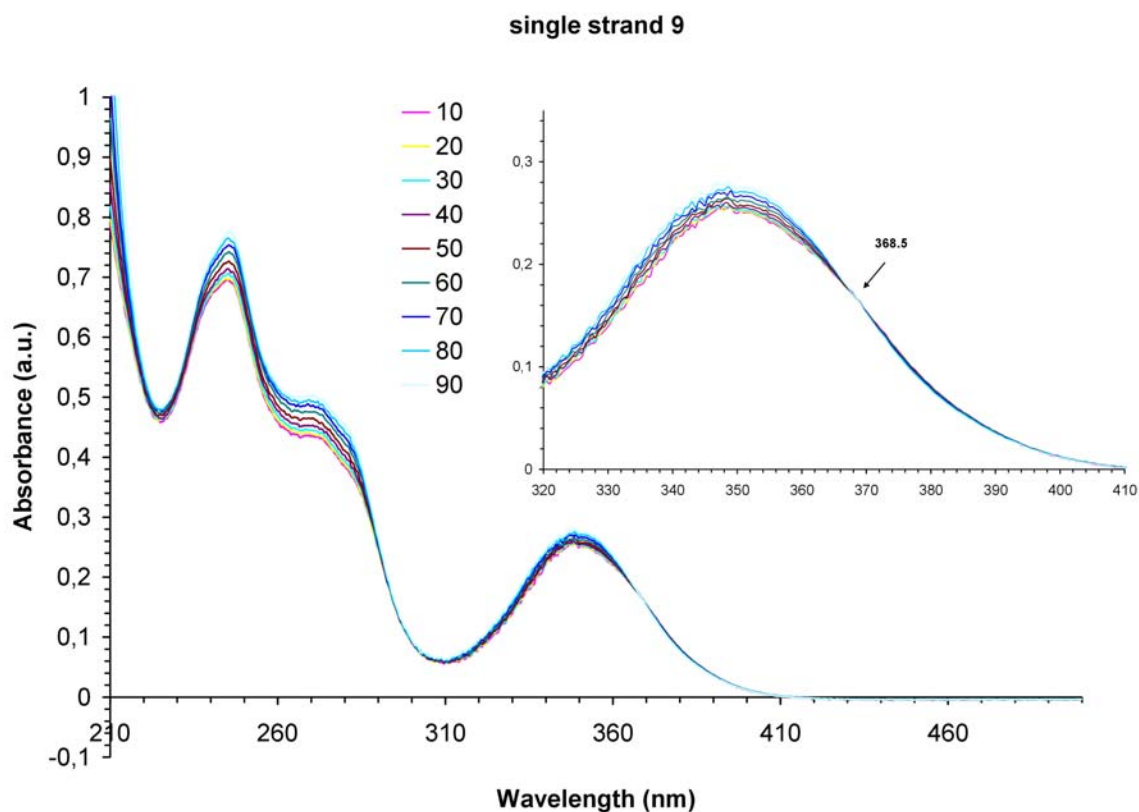


Figure 2. Temperature-dependent UV-Vis of single strand **9** (210-500 nm, 10-90 °C)

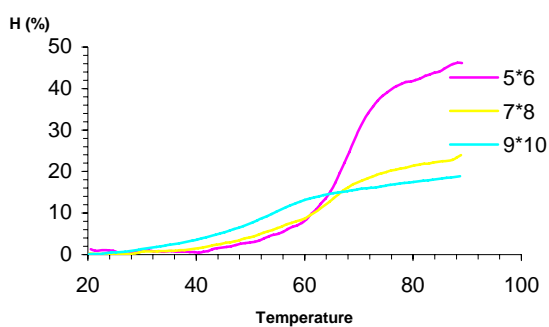


Figure 3. Hyperchromicity of duplexes at 354 nm.

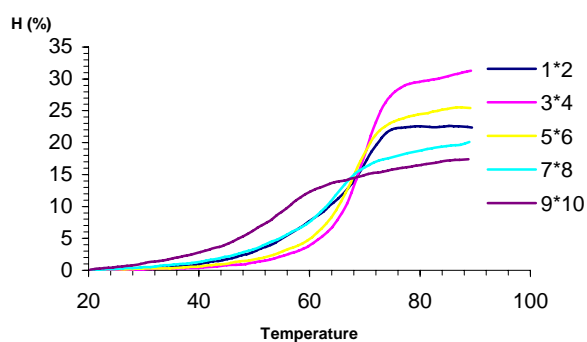


Figure 4. Hyperchromicity at 245 nm.

H at 80 °C	1*2	3*4	5*6	7*8	9*10
H ₂₄₅	22.6	29.6	24.4	18.7	16.4
H ₃₅₄			42.1	21.5	17.4
H ₃₅₄ per pyrene			10.5	3.6	1.2

Note: notable decrease of observed hyperchromicity per pyrene unit (H₃₅₄ per pyrene) with increasing of pyrenes within hybrid (**5*6**, **7*8** and **9*10**) strongly supports high degree of pyrene pre-aggregation even at high temperature.

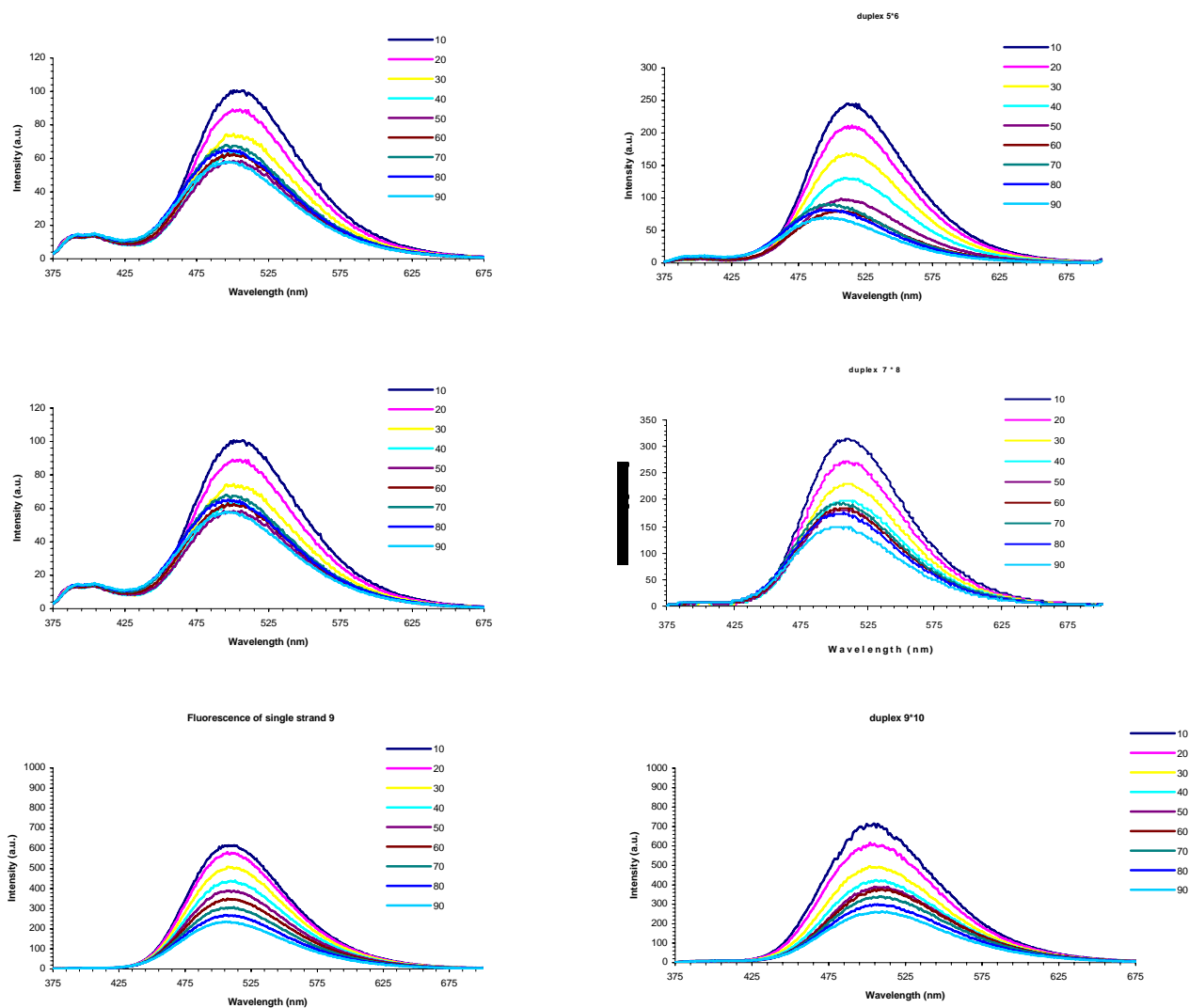


Figure 5. Fluorescence spectra of single strands a) 5; b) 7; c) 9 (left column) and duplexes d) 5*6, e) 7*8, f) 9*10 (right column)

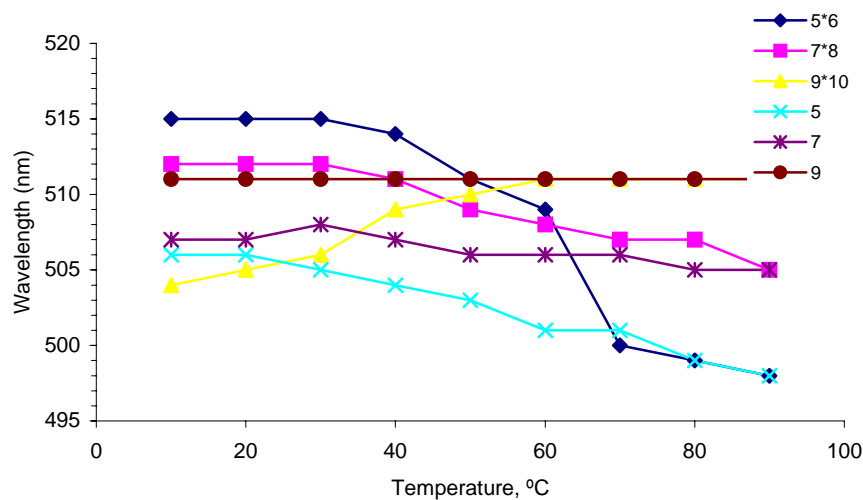


Figure 6. Trends of fluorescence maximum shifts upon melting of duplexes 5*6, 7*8, 9*10 and single strands 5, 7, 9.

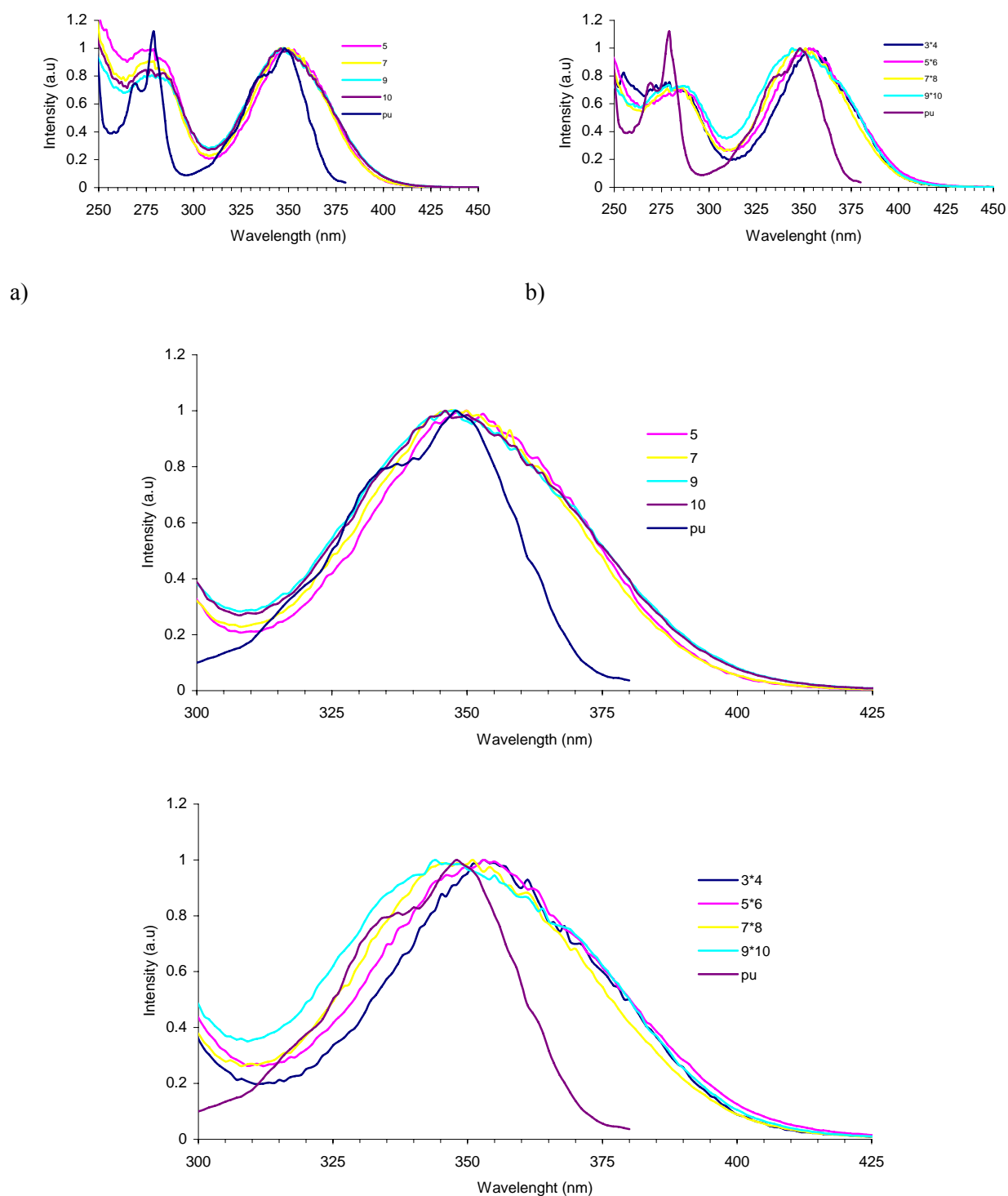


Figure 7. Normalized excitation spectra a) single strands **5**, **7**, **9**, **10** and pyrene-1,8-dicarboxylic acid bis-[(3-hydroxy-propyl)amide] (PU, used as reference of monomeric pyrene), and b) hybrids **3*4**, **5*6**, **7*8**, **9*10**.

Note: broadening of excitation spectra compared to monomeric pyrene (PU) upon increasing number of pyrene per single strand is clearly observed (**5**, **7** and **9**, respectively). In addition, duplex formation is accompanied with a further increase of this broadening. The broadening is accompanied with a decrease of the Intensity Peak/Intensity Valley ratio, which also serves as a qualitative indication on the extent of pyrene aggregation (see: F. M. Winnik, *Chem. Rev.* **1993**, 587-614).

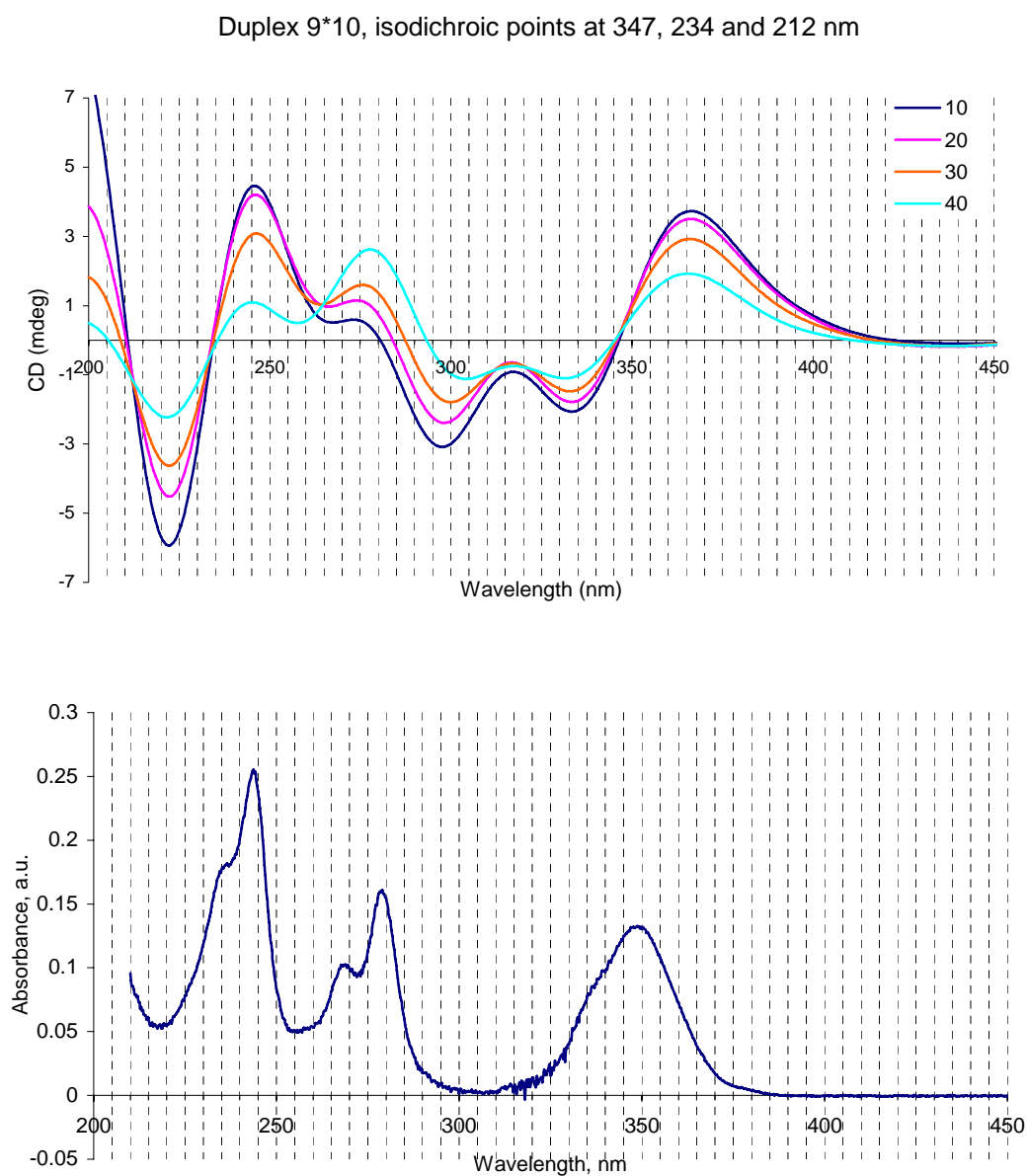


Figure 8. a) Isodichroic points for duplex **9*10** (10-40 °C); b) UV-Vis spectrum of the pyrene building block (10⁻⁶ M) in phosphate buffer solution (pH = 7.0) at 20 °C.

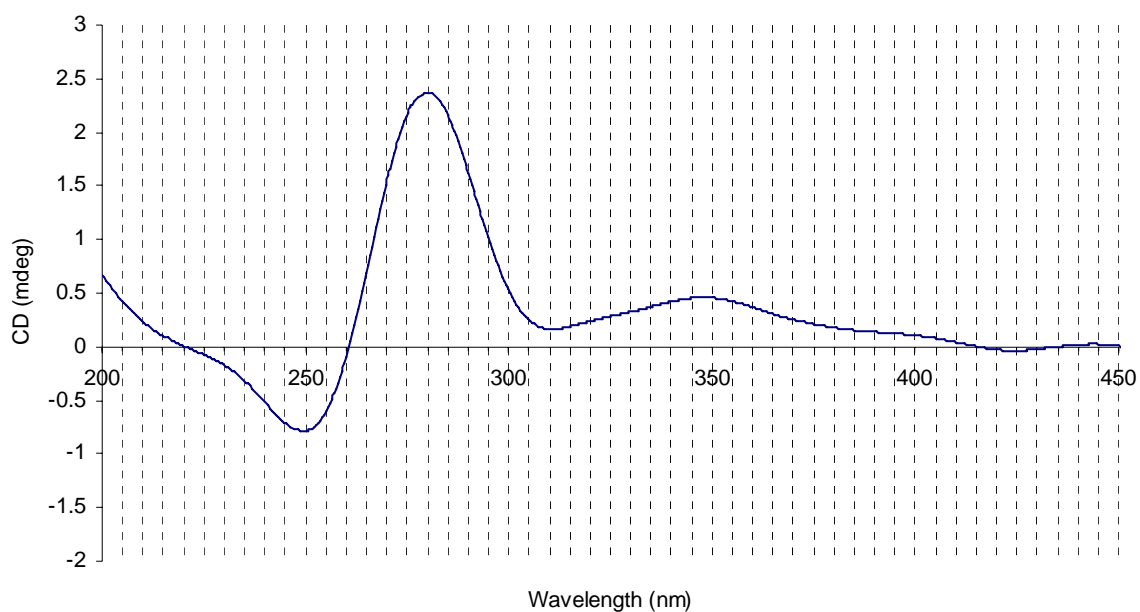


Figure 9. Example of CD spectra of single strand **9** (1.0 μ M, pH 7, 10 $^{\circ}$ C).

Note: The band at 350 nm corresponding to the maximum of pyrene absorbance is not split. Therefore it represents ICD (induced CD) of pyrene induced by chiral environment of the oligonucleotides.

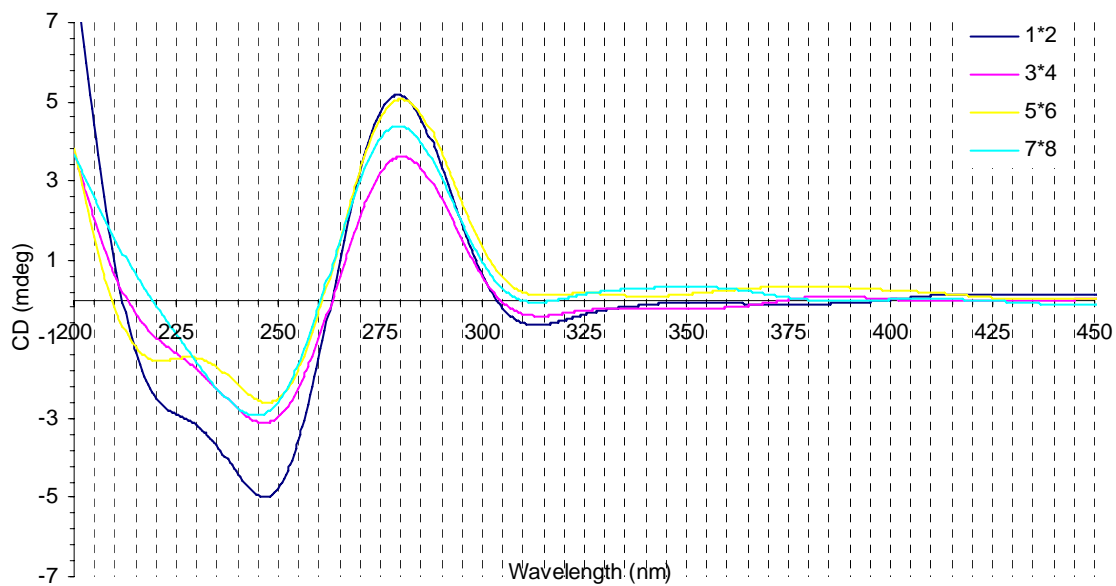
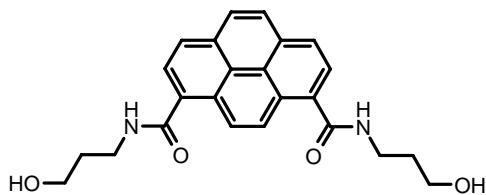


Figure 10. CD spectra of duplexes **1*2**, **3*4**, **5*6** and **7*8**.

4) X-ray crystallography



Pyrene-1,8-dicarboxylic acid bis-[(3-hydroxy-propyl)amide]

A light yellow crystal of pyrene-1,8-dicarboxylic acid bis-[(3-hydroxy-propyl)amide] was mounted on a Stoe Mark II-Image Plate Diffraction System.¹ The intensity data were collected at 173K (-100°C) using MoK α graphite monochromated radiation, image plate distance 100mm, ω oscillation scans 0 - 180° at ϕ 0°, 2 θ range 2.29 – 59.53°, d_{\max} - d_{\min} = 17.78 - 0.72 Å.

The compound crystallises in the centrosymmetric monoclinic space group C2/c, the molecular formula is [C₂₄H₂₄N₂O₄].

The structure was solved by direct methods using the programme SHELXS-97.² The refinement and all further calculations were carried out using SHELXL-97.³ All hydrogen atoms were included in calculated positions and treated as riding atoms using SHELXL-97 default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F². The program PLATON was used for molecular drawings.⁴ No absorption correction was applied.

- [1] Stoe & Cie (2002). *X-Area VI.17 & X-RED32 VI.04 Software*. Stoe & Cie GmbH, Darmstadt, Germany.
- [2] G. M. Sheldrick, (1990) "SHELXS-97 Program for Crystal Structure Determination", *Acta Crystallogr.*, **A46**, 467-473.
- [3] G. Sheldrick, (1999) "SHELXL-97", Universität Göttingen, Göttingen, Germany.
- [4] Spek, A. L. (2003). *J.Appl.Cryst.* **36**, 7-13.

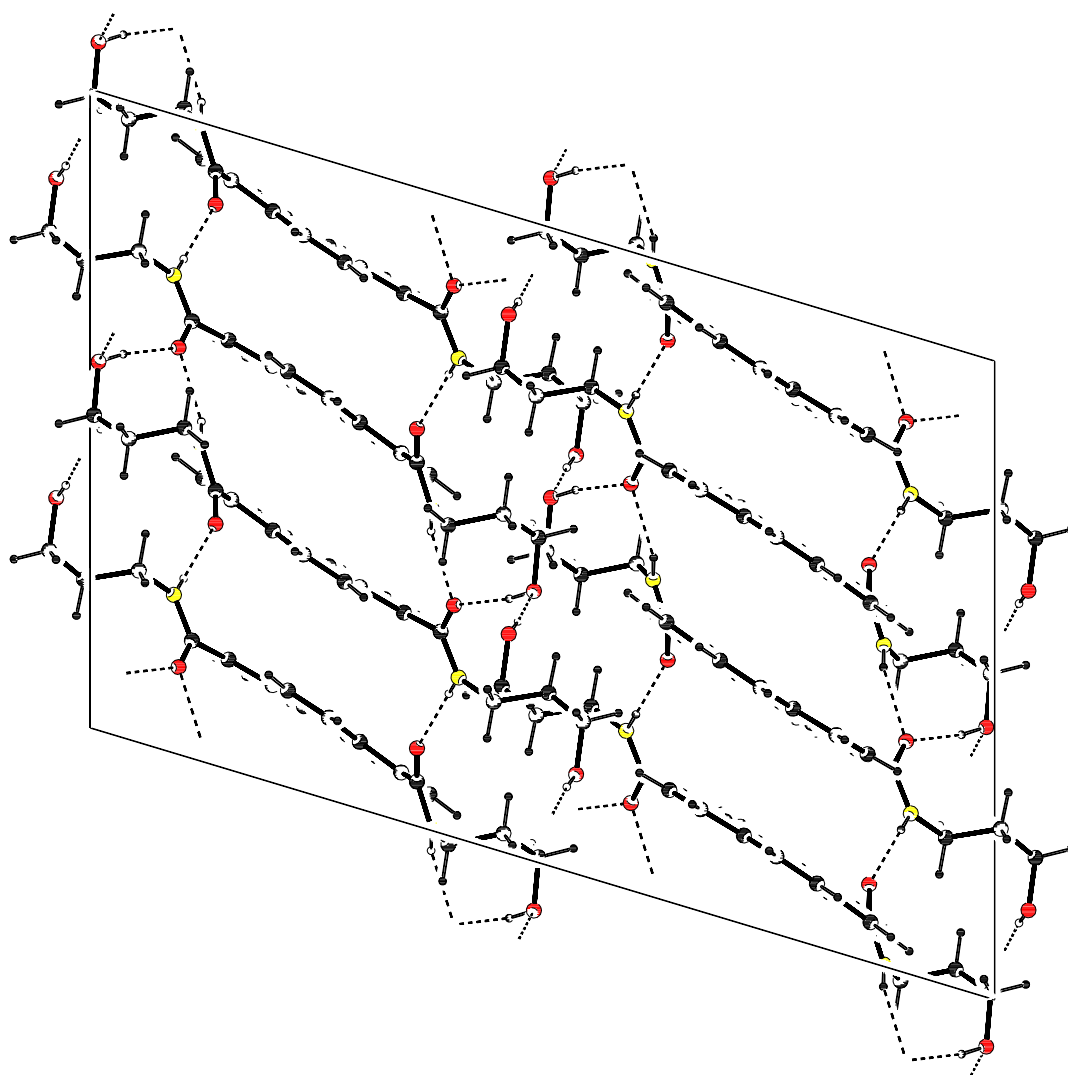


Figure 11. Packing diagram showing hydrogen bonds (N – yellow, O – red),
made by A. Neels, University of Neuchatel, Switzerland.

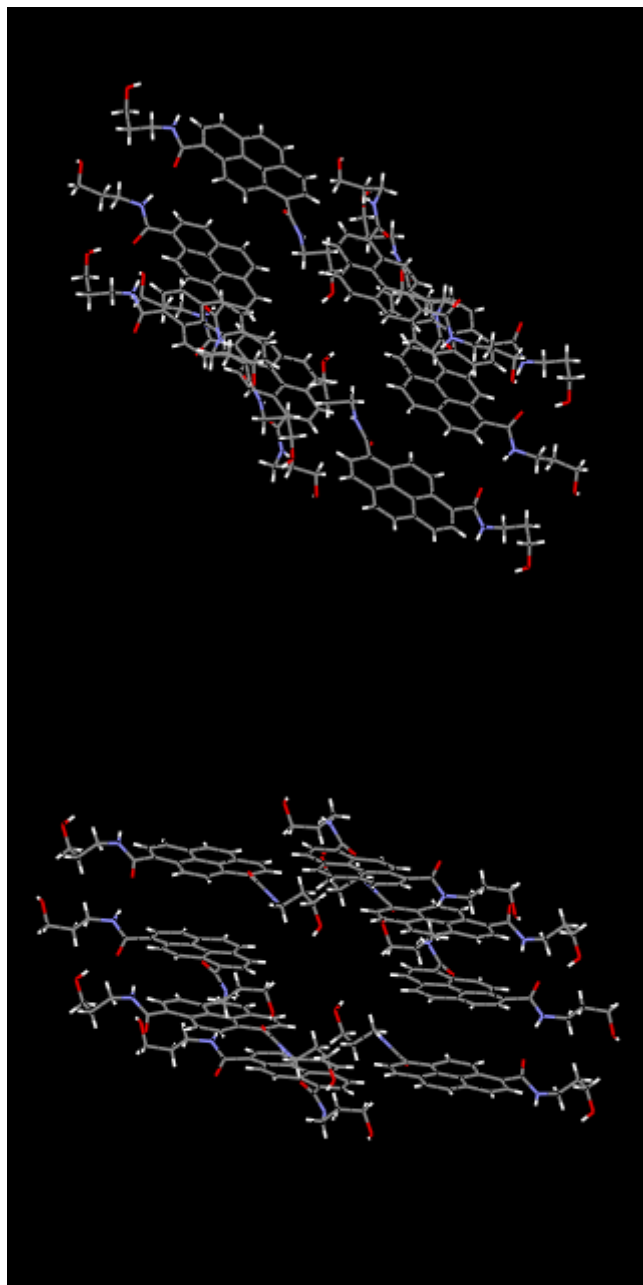


Figure 12. Representative pictures of X-ray for the pyrene building block.