Spicing up an Interdisciplinary Chemical Biology Course with the Authentic Big Picture of Epigenetic Research

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ABBREVIATIONS

APS	ammoniumperoxodisulfate
bp	base pair
CD	circular dichroism
CE	capillary electrophoresis
ddH2O	double distilled water
dNTP	nucleoside triphosphate
EDTA	ethylenediaminetetraacetic acid
FAM	fluorescein
FID	fluorescence intercalation displacement
Fmoc	fluorenylmethoxycaarbonyl
HATU	[4,5-b]pyridinium 3-oxid hexafluorophosphate
HF	high fidelity
ICD	induced circular dichroism
Im	imidazole
K_D	binding constant
NoSP	nature of scientific writing
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
Py	pyrrole
RP-HPLC	reverse phase high performance liquid chromatography
SDB	salt dialysis buffer
SPPS	solid phase peptide synthesis
TBE	Tris-Borate-EDTA buffer
TEMED	tetramethylethylenediamine
test cl.	test cleavage
TO	thiazole orange
W601	Widom 601 DNA

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COURSE TIMETABLES AND TOPIC DESCRIPTIONS

The timetables show the plan of the whole course (Table S1) and the specific topics covered during the scientific communication workshop (Table S2). The lecture topics are shown in the manuscript in Box 1 (Figure 2).

Table S1. General course timetable.

time	*duration*	*event*	*description*	*assignment(s) (outcome/observation)*
week -10	1h	information meeting	basic description of the course to interested master students from chemistry and biology	
week -3		sign up deadline	students had to sign up for the course	signing in
week 1	2h	lecture 1	topic L1	
week 2	2h	lecture 2, workshop 1	topic L2, topic W1	
week 3	2h	Pomodoro 1	group formation for research proposal, rounds of brainstorming and group feedback I	brainstorming and topic idea finding in groups
week 4	2h	lecture 3, workshop 2	topic L3, topic W2	
week 5	2h	lecture 4	topic L4	
week 6	2h	Pomodoro 2	rounds of brainstorming and group feedback II	group work on proposal abstract
week 7	2h	lecture 5, workshop 3	topic L5, topic W3	
week 8	full days	lab course week 1	introduction, security introduction, lab exercises (see extra lab course plan)	hand in proposal abstract
	2h	lecture 6, workshop 4	topic L6, topic W4	
week 9	full days	lab course week 2	lab exercises (see extra lab course plan)	
	2h	workshop 5	topic W5	
	1h	scientific writing	students received group feedback about submitted proposal abstract	
week 10	2h	workshop 6	topic W6 by an Scientific English expert	
week 10	flexible	scientific writing	communication	individual writing of communication
week 11	flexible	scientific writing	communication	individual writing of communication
week 12	flexible	scientific writing	poster of proposal	hand in communication, group design of poster about proposal
week 13	flexible	scientific writing	poster of proposal, group feedback on draft poster version	hand in draft of the poster version, group work on final poster about proposal
week 14	2h	final presentation	poster presentation and individual feedback by external referees	group poster presentation
	flexible	feedback	feedback of the communication	

Table S2. Course material covered during the scientific communication workshops.

topic	*topic description*
topic W1	Overview: types of scientific writing; structure; writing elements by analyzing articles
topic W2	Drafting: importance of the outline; practical exercises abstract, title and keywords; practical exercises
topic W3	From introduction to conclusions
topic W4	Referring an article and in-class writing exercises
topic W5	Effective use of figures and tables
topic W6	Use of English by a Scientific English expert

DISCOVERY-BASED RESEARCH LAB DESIGN

Tables S3 and S4 summarize a detailed plan for each day of the discovery-based research lab. Table S5 shows the parameters, which were on the research question and experimental techniques to answer them.

Table S3. Plan of the first week for the discovery-based research lab.

group	*Monday*	*Tuesday*	*Wednesday*	*Thursday*	*Friday*
A1: monomer	get glassware, start acetylation of Py + Im	purify acetylation of Py + Im start nitration of Py + Im	purify nitration of Py + Im start esterification of Py + Im	purify esterification of Py	purify esterification of Im
B1: hairpin	get glassware, learn SPPS start hairpin synthesis	continue hairpin synthesis, test cleavage	analyze test cl. continue hairpin synthesis, test cleavage	analyze test cl. continue hairpin synthesis, test cleavage	analyze test cl. final cleavage and purification
C1: nucleosome	get glassware , prepare buffers	PCR W601, gel for purity	start nucleosome test assembly	check nucleosome purity, nucleosome big scale assembly	check nucleosome purity

Table S4. Plan of the second week for discovery-based research lab.

group	*Monday*	*Tuesday*	*Wednesday*	*Thursday*	*Friday*
A2: analysis of hairpins by CD	presentation meeting 1 finish rest of 1. week introduction in all new devices	CD measurement	CD measurement	analysis of data	presentation meeting 2 finish rest, clean all give glassware back
B2: analysis of hairpins by FID	presentation meeting 1 finish rest of 1. week introduction in all new devices	fluorescence displacement measurement	fluorescence displacement measurement	analysis of data	presentation meeting 2 finish rest, clean all give glassware back
C2: analysis of sequence specific nucleosome binding (footprinting)	presentation meeting 1 finish rest of 1. week introduction in all new devices	prepare sample	CE measurement	analysis of data	presentation meeting 2 finish rest, clean all give glasware back

Table S5. Parameters, which were on the research question and experimental techniques to answer them.

Parameter (research question)	*Experimental technique (answer)*
Binding affinity to free DNA	CD spectroscopy (induced circular dichroism) fluorescence spectroscopy (fluorescence intercalation displacement) capillary electrophoresis
Binding affinity to nucleosomes	capillary electrophoresis

LIST OF REAGENTS

Table S6 summarizes the used reagents, including its corresponding CAS-number and the used supplier. For non-commercial reagents, procedures and references for preparations are given.

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Table S6. List of used reagents including their CAS-number and the used supplier.

Chemical	*CAS-number*	*supplier*
trichloroacetyl chloride	76-02-8	Sigma Aldrich (USA)
N-methylpyrrole	96-54-8	Acros Organics (Belgium)
potassium carbonate	584-08-7	Carl Roth (Germany)
magnesium sulfate anhydrous	7487-88-9	Carl Roth (Germany)
acetic anhydride	108-24-7	Carl Roth (Germany)
conc. Nitric acid	7697-37-2	Merck (Germany)
sodium carbonate	497-19-8	Carl Roth (Germany)
sodium chloride	7647-14-5	VWR (USA)
4-dimethylaminopyridine (DMAP)	1122-58-3	Fluka (USA)
N-methylimidazole	616-47-7	Sigma Aldrich (USA)
triethylamine	121-44-8	VWR (USA)
sodium hydrogen carbonate	144-55-8	Carl Roth (Germany)
dimethylformamid (DMF)	68-12-2	Iris Biotech (Germany)
N-methyl-2-pyrrolidone (NMP)	872-50-4	Iris Biotech (Germany)
N,N-diisopropylethylamine (DIPEA)	7087-68-5	Carl Roth (Germany)
1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU)	148893-10-1	Iris Biotech (Germany)
Rink amide (RAM) MBHA-resin	431041-83-7	Fluorochem (UK)
Fmoc-βAla-OH	35737-10-1	TCI (Japan)
Fmoc-γ-OH	116821-47-7	Iris Biotech (Germany)
piperidine	110-89-4	Iris Biotech (Germany)
pyridine	110-86-1	VWR (USA)
DMSO biomolecular grade	67-68-5	Sigma Aldrich (USA)
triisopropylsilane (TIS)	6485-79-6	Sigma Aldrich (USA)
trifluoroacetic acid (TFA)	76-05-1	abcr (Germany)
5XHF buffer	-	New England Biolabs (USA)
dNTPs	-	Invitrogen (USA)
primers	-	Sigma Aldrich (USA)
phusion polymerase	-	New England Biolabs (USA)
40% 19:1 acrylamide : bisacrylamide	-	Carl Roth (Germany)
tetramethylethylenediamine (TEMED)	110-18-9	Carl Roth (Germany)
ammoniumperoxodisulfate (APS)	7727-54-0	Carl Roth (Germany)
tris(hydroxymethyl)aminomethane (Tris)	77-86-1	Carl Roth (Germany)
ethylenediaminetetraacetic acid (EDTA)	60-00-4	Merck (Germany)
boric acid	10043-35-3	Carl Roth (Germany)
sodium dihydrogen phosphate monohydrate	10049-21-5	Merck (Germany)

thiazole orange (TO)	107091-89-4	Sigma Aldrich (USA)
hairpin DNAs	-	Sigma Aldrich (USA)
Fmoc-βAla-RAM resin	Load RAM-MBHA-resin following reference [1]	
Fmoc-Py-OH	Synthesized following reference [2,3]	
Fmoc-Im-OH	Synthesized following reference [2,3,4]	
Fmoc-Im-Py-OH	Synthesized following reference [5]	
plasmid pGEM-3z/601	Addgene plasmid #26656, deposited by Jonathan Widom	
chicken erythrocyte histone octamers	Prepared following reference [6]	

HAZARDS

Within each lab assignment the hazards varied. Especially in the synthesis stations, the most common hazards were flammable liquids and toxic reagents, which were reduced to a minimum amount and replaced when alternatives were possible. All hazardous work was performed in fume hoods and protective coats and googles were compulsory during the whole two weeks of the practical course. Before the course, all students received a basic security introduction considering the inexperience of the biologists. The main security issues were highlighted and hazardous steps were mentioned and discussed during the course too.

PRIMER SEQUENCES AND DNA PREPARATION

Table S7 shows the primer sequences that were used in the polymerase chain reaction of Widom 601 DNA.

Table S7. Primer sequences used in the polymerase chain reaction of Widom 601 DNA.

primer	*Sequence 5'-3'*
forward primer FAM601_F	[6FAM] CCT GGA GAA TCC CGG TGC
reverse primer 601_R	CAG GAT GTA TAT ATC TGA CAC GTG CC

The DNA hairpins for the CD-spectroscopy and fluorescence intercalation displacement (FID) were prepared prior to the laboratory course. The two DNA strands from Table S8 were dissolved in ddH_2O and each mixture was heated to 95 °C for 10 min and then slowly cooled to r.t. Their concentration was determined spectroscopically on a Tecan Spark 20M. Their molar extinction coefficients were determined by using the following formula:[7]

$$\varepsilon_{260nm} = \{(8.8 * \#T) + (7.3 * \#C) + (11.7 * \#G) + (15.4 * \#A)\} * \ 0.8 * \ 10^3 \ M^{-1}cm^{-1} \quad \text{(S1)}$$

In the formula # = number of nucleobases determined throughout the DNA sequence, T = thymine, C = cytosine, G = guanine, A = adenine.

Table S8. DNA sequences used for the CD and FID analysis.

DNA name	*DNA sequence 5'-3'*
Nucleosome_1	GGC AGTGTA CGC TTTTT GCG TACACT GCC
Nucleosome_2	GGC AGACTA CGC TTTTT GCG TAGTCT GCC

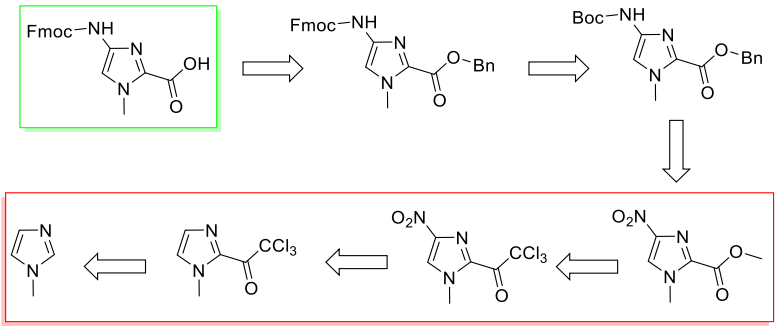
EXPERIMENTAL DESCRIPTION FOR EACH GROUP

Week 1, group A1 – Pyrrole (Py) and imidazole (Im) monomer synthesis:

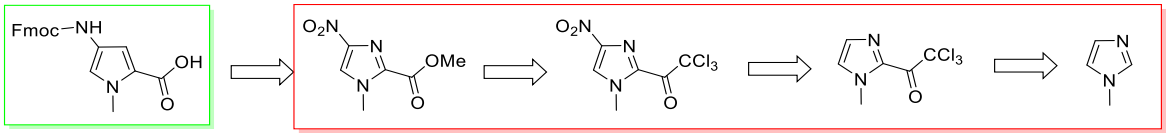
References: *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 6864.; *Org. Lett.* **2001**, *3*, 1201.; *J. Am.Chem. Soc.* **1996**, *118*, 6141.; *J. Am.Chem. Soc.* **2006**, 8766.; *Heterocycles* **1988**, *27*, 1945.; Patent WO 199703975 A2.

Questions: Why do we need to go two different ways of synthesis for the imidazole and the pyrrole monomer? What do we need all the chemicals in each reaction for? What are the mechanisms of the reactions? With which chemicals do we need to work carefully?

Retrosynthetic pathway of the monomer synthesis:



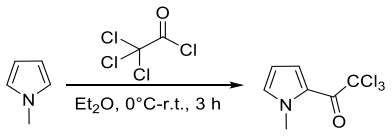
Scheme S1. Retrosynthetic pathway for the imidazole monomer. Synthesis steps which were performed by the students are highlighted with a red box. The final green-highlighted Fmoc-monomer was previously prepared and supplied by the instructors.



Scheme S2. Retrosynthetic pathway for the pyrrole monomer. Synthesis steps which were performed by the students are marked with a red box. The final green-highlighted Fmoc-monomer was previously prepared and supplied by the lab instructors.

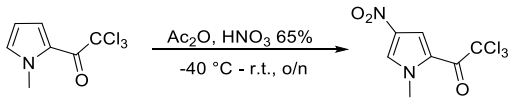
Below, the detailed synthetic procedures for the synthesis steps which were performed by the students is shown. The remaining steps until the final Fmoc-building blocks were conducted and supplied by the instructors, due to time restrictions and dangerous and complicated synthesis steps. The references which were used to prepare the final monomers are summarized in Table S6.

Synthesis of Py-C(O)CCl_3



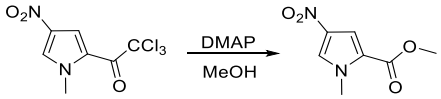
Under nitrogen atmosphere, trichloroacetylchlorid (50.0 mmol) was dissolved in Et_2O (10 mL) and cooled to 0 °C. *N*-Methylpyrrole (50.0 mmol) was dissolved in Et_2O (10 mL) and added dropwise over 30 min. The reaction mixture was warmed up to r.t. and stirred for another 3 h. At 0 °C, the reaction was quenched with K_2CO_3 (26 mmol) dissolved in H_2O (14 mL). It was extracted with Et_2O, dried over $MgSO_4$, filtered and the solvent removed. Analysis was performed (^1H, ^{13}C, CI).

Synthesis of NO_2-Py-C(O)CCl_3



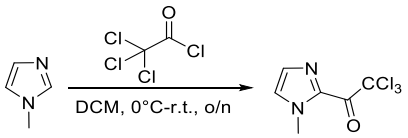
Under nitrogen atmosphere, Py-C(O)CCl_3 (1 eq) was dissolved in Ac_2O (13 eq) and cooled down to -40 °C. 65% HNO_3 (2.2 eq) was added dropwise. The reaction mixture was slowly warmed up to r.t. and stirred overnight. At 0 °C, the reaction mixture was carefully neutralized with Na_2CO_3 (sat.) to pH 7. It was extracted 4 x with EtOAc and washed 3 x with brine. The organic layers were dried over $MgSO_4$, filtered and the solvent removed. Analysis was performed (^1H, ^{13}C, CI).

Synthesis of NO_2-Py-OMe



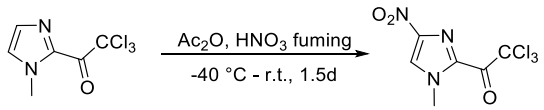
NO_2-Py-C(O)CCl_3 (1 eq) was dissolved in MeOH to give a 0.3 M solution. DMAP (0.1 eq) was added and the solution stirred overnight. The solvent was removed in a dryload and the crude purified by flash column chromatography (pentane/EtOAc 4:1 → 1:1). Analysis was performed (^1H, ^{13}C, ESI^+).

Synthesis of Im-C(O)CCl_3



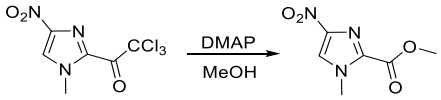
Under nitrogen atmosphere, trichloroacetylchlorid (35.1 mmol) was dissolved in CH_2Cl_2 (25 mL) and cooled to 0 °C. *N*-Methylimidazole (35.0 mmol) was dissolved in CH_2Cl_2 (15 mL) and added dropwise over 1.5 h. The reaction mixture was warmed up to r.t. and stirred until full conversion (at least 3 h). At 0 °C, freshly destilled NEt_3 (35.1 mmol) was added dropwise over 30 min. The solvent was removed in a dryload and the crude purified by flash column chromatography (pentane/EtOAc 1:1). Analysis was performed (^1H, ^{13}C in $CDCl_3$, CI).

Synthesis of NO_2-Im-C(O)CCl_3



Under nitrogen atmosphere, Im-C(O)CCl_3 (1 eq) was dissolved in Ac_2O (13 eq) and cooled down to -40 °C. Fuming HNO_3 (6.5 eq) was added dropwise over 1.5 h. The reaction mixture was slowly warmed up to r.t. and stirred overnight. The reaction mixture was neutralized carefully with saturated $NaHCO_3$ under cooling and extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and the solvent removed. Analysis was performed (^1H, ^{13}C in $CDCl_3$, CI).

Synthesis of NO_2-Im-OMe



Under nitrogen atmosphere, NO_2-Im-C(O)CCl_3 (1 eq) was dissolved in MeOH to give a 0.3 M solution. DMAP (0.1 eq) was added and the solution stirred overnight. The solvent was removed in a dryload and the crude purified by flash column chromatography (pentane/EtOAc 4:1 → 1:1 → 1:4). The gradient was not increased too fast, as with this column the 4-NO_2- and 5-NO_2-isomer were separated. Analysis was performed (^1H, ^{13}C in DMSO, ESI^+).

Week 1, group B1 - Polyamide Hairpin Synthesis:

General procedure for the polyamide hairpin coupling

The hairpins were synthesised manually in a 20 µmol scale in 2 mL polypropylene syringes equipped with a filter and plunger (pore size 25 µm, MultiSynTech GmbH; Germany). All solutions needed were freshly prepared in the morning of use.

The needed amount of preloaded Fmoc-βAla-RAM-resin (loading = 0.54 mmol/g) was weighted into a filter-syringe and the resin swelled in 1.5 mL DMF for 30 min.

Solutions were prepared in 50 mL falcon tubes and refilled to 1.5 mL reaction tubes:

deprotection solution: 20% piperidine in DMF, 500 µL (24x) /1.5 mL reaction tubes (12 mL in total)

capping solution: 5% Ac_2O, 5% pyridine in DMF, 500 µL (12x) /1.5 mL reaction tubes (6 mL in total)

It was followed the procedure of the table at the end of this protocol:

deprotection: Deprotection was performed twice for 5 min.

coupling: Coupling **of Fmoc-Py-OH** was performed with 4 eq of the amino acid and 4 eq HATU dissolved in **DMF** to give a 0.3 M solution. DIPEA (8 eq) was added and the mixture preincubated for 3 min before addition to the resin. Coupling was performed for 1 h. After introduction of Fmoc-γ-OH, after 1 h of coupling, DMSO/DMF 1:1 (100 µL) was added for 30 min.

Coupling **of Fmoc-Im-Py-OH** and **Fmoc-Im-OH** was performed with 4 eq of the amino acid and 4 eq HATU dissolved in **NMP** to give a 0.3 M solution. DIPEA (12 eq) was added and the mixture preincubated for 3 min before addition to the resin. Coupling was performed for 2 h. After introduction of Fmoc-γ-OH, after 2 h of coupling, DMSO/DMF 1:1 (100 µL) was added for 30 min.

capping: Capping was performed once for 5 min.

test cleavage: After the capping step, a little sample of resin, which was washed with CH_2Cl_2 last, was transferred to an 1.5 mL reaction tubes and 180 µL cleavage mixture (TFA/CH_2Cl_2/H_2O/TIS 90/5/2.5/2.5) were added and shacked at r.t. for 1.5 to 2 h. The solvent was transferred into another 1.5 mL reaction tube without the resin balls, the solvent was removed under nitrogen flow, and the crude dried under vacuum for 10 min. The sample was re-dissolved in H_2O/MeCN 7/3 0.1% TFA and the OD_{304} was determined on the nanodrop and the sample injected in the HPLC to verify the successful couplings. The injected amount was calculated by V = 3.6 µL / OD_{304}.

final cleavage: To the resin, cleavage mixture (1 mL /10 μmol, TFA/CH_2Cl_2/H_2O/TIS 90/5/2.5/2.5) was added and stirred at r.t. for 2 h. The solvent was transferred into a filter syringe, filtered and washed once with 1 mL TFA, to remove the resin balls. The solvent was removed under nitrogen flow and the crude dried under vacuum for 10 min. The crude was dissolved in H_2O/MeCN (7/3) 0.1% TFA and purified on the preparative RP-HPLC.

Synthesize the two following hairpins:

- Nucleosome 1: Ac-Im-Py-Im-Py-γ-Py-Py-Py-Py-β-NH_2 (20 μmol) (targets the α-satellite nucleosome)
- Nucleosome 2: Ac-Im-Py-Py-Py-γ-Py-Im-Py-Py-β-NH_2 (20 μmol) (targets the W601 nucleosome)

m /mg	Fmoc-aa	Deprotection 2 x 5min 500μL		Wash 5xDMF,5xDCM, 5xDMF	Cap/ change needle	Coupling 1h /2h	DMSO addition 30 min	Wash 5xDMF	Capping 5 min 500μL	Wash 5xDMF, 5xDCM, 5xDMF

Pairing rules for Dervan-polyamide hairpins

Pairing rules for polyamides, which bind sequence-specific to the DNA, were nicely summarized by Dervan and Bürli.[8] By designing the right polyamide, many different sequences can be addressed. For the design of a sequence, some rules needs to be followed: a pyrrole opposite of an imidazole (Py/Im) targets a C-G basepair, whereas an imidazole opposite of a pyrrole (Im/Py) targets a G-C basepair. A pyrrole pyrrole pair (Py/Py) targets both, A-T and T-A. The C-terminal β-alanine linker and the γ-turn both are specific for an A/T or T/A pair on the flanking sides of the polyamide. β-alanine can be introduced instead of pyrrole and reveals the same binding selectivity's. However, their introduction gives a less rigid polyamide hairpin, which results in less truncations during coupling and therefore higher yields. The orientation of the polyamide upon binding to the DNA orientates the *N*→*C* of the polyamide in the 5'→3' direction of the DNA. The table below summarizes the pairing rules of all building block combinations. The example polyamide in the figure below illustrates its pairing rules to a 6-bp DNA.

Table S9. Pairing rules of all building block combinations for the synthesis of polyamides.

pair	G-C	C-G	T-A	A-T
Im/Py	+	-	-	-
Py/Im	-	+	-	-
Py/Py	-	-	+	+
Im/Im	-	-	-	-
Im/β	+	-	-	-
β/Im	-	+	-	-
Py/β	-	-	+	+
β/Py	-	-	+	+
β/β	-	-	+	+

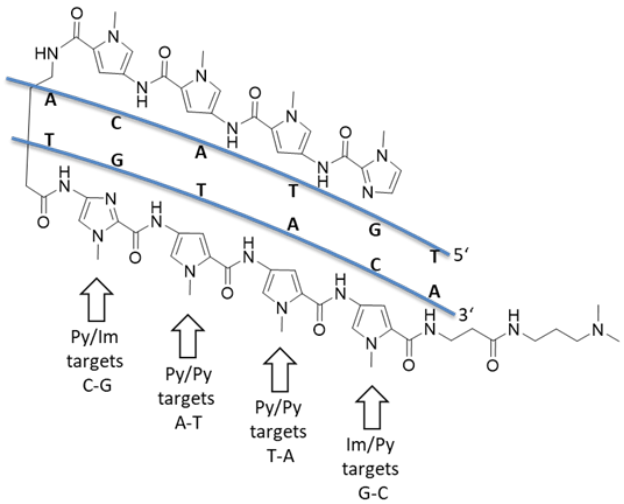


Figure S1. Example of polyamide hairpin binding to a 6 bp DNA to visualize the pairing rules.

Week 1, group C1 - Nucleosome Assembly:

PCR for DNA601 and FAM-DNA601:

Questions: What does PCR stands for? How does PCR works? What do we need each component of the PCR for? How does the purification of the produced DNA work?

Procedure for W601-DNA:

During the PCR, all components were **always** stored on ice. The phusion polymerase was stored in the cooling block and only shortly taken out of the freezer when needed.

	amount [µL]	final concentration
ddH_2O	532.2	
5xHF	160	1X
DMSO 100%	24	3%
dNTP(10 mM)	16 each	200 µM each
primer 601_R (HPLC, 100 µM)	4	0.5 µM
primer FAM601_F (100 µM)	4	0.5 µM
plasmid pGEM-601 (211 ng/µL)	3.8	1 ng/µL
phusion polymerase (2 U/µL)	8	1 U/ 50 µL

The mixtures were prepared twice. All components except the phusion polymerase were combined and mixed gently with a pipette. The mixture was stored on ice and it was checked for the free PCR cycler. At last, the phusion polymerase was added, mixed again gently and portions of 50 µL were filled in each PCR tube. All the liquid was shortly spinned down and placed into the PCR cycler. The program of the table below was started:

98 °C	30 s
98 °C	8 s
60 °C	20 s
72 °C	10 s
72 °C	4 min
4 °C	hold

After the program was finished, two PCR tubes were combined in one 1.5 mL reaction tubes and the procedure of the *PCR & DNA Clean Up Kit* (New England Biolabs) provided was followed, but 600 µL loading buffer used and the columns loaded twice. 5 columns in total were used.

The DNA was eluted from the column with 10 µL ddH_2O and all eluents collected in one 1.5 mL reaction tube. It was re-eluted with 25 µL elution buffer. Afterwards, the DNA concentration was determined on the nanodrop.

Determination of DNA purity

To determine the purity of prepared DNA, a 2% agarose gel was run. To prepare the gel, 1.2 g of agarose was added to 60 g of 1X TBE buffer in an Erlenmeyer flask. The mixture was heated until all particles were completely dissolved and the solution was gently boiling. The solution was cooled down for 3 min and 2 µL of midori green were added. It was shaked again gently, poored into a gel caster and the comb placed into it. After polymerisation was finished the comb was removed carefully and the gel transferred into the running chamber filled with 1X TBE and the pockets were filled with:

1) 5 µL of DNA ladder CSL 1kb

2) 0.3 µg of the prepared DNA in a total volume of 6 µL TBE buffer and 1 µL 6x DNA loading dye

The gel was run for 40 min at 90 V. The gel was analyzed with a ChemiDoc MP by Biorad.

Nucleosome assembly:[9]

Questions: Which of the used chemicals are dangerous? Are all nucleosomes the same? – check for alpha-satellite, widom601 and the composition of the histone core. What are PTMs? How does the reconstitution of nucleosomes work?

Buffer preparation

Nucleosome Core Particles (NCPs) were reconstituted by the salt-gradient-dialysis method. Different buffers were prepared:

1 L 20X TE buffer: 200 mM Tris, 20 mM EDTA, pH 8.0

1 L 10X TBE buffer: 40 mL 0.5 M EDTA pH 8.0, 108 g Tris, 55g boric acid → filled up to 1 L

250 mL SDB buffer ("salt dialysis buffer"): 10 mM Tris, 1 mM EDTA, 2 M NaCl, pH 8.0
(12.5 mL 20X TE, 29.22g NaCl → filled up to 125 mL, took out 1 mL and filled up to a total volume of 250 mL)

1L of 1X TE-buffer (10 mM Tris, 1 mM EDTA, pH 8.0)

All buffers were stored at 4°C

Reconstitution of NCPs

It was reconstituted in a volume of 30 µL, which contained 1 µg of DNA (90876 Da) and varying amounts of histone octamers prepared from chicken erythrocytes. To assemble this 1 µg of DNA, about 2.4 µL of the 1:10 glycerol stock of histones in 1X SDB were needed. It was planned to prepare a titration row with 11.0 / 11.5 / 12.0 / 12.5 µL of the 1:50 stock to determine the exact ratio needed.

The dialysis membrane was placed in 1X SDB+2M NaCl for at least 20 min.

The used histone dilution in low binding tubes was: 1:50 glycerol stock in 1X SDB
Histone and DNA mixtures were prepared in low binding tubes by adding 1.0 µg DNA and the volumes given above of the 1:50 histone stock into a total volume of 30 µL SDB

Dialysis chambers: Samples were pipetted into cut 1.5 ml reaction tube caps and a small membrane was added (The membrane was placed in water before and shortly put into the beaker (2 M NaCl). The membranes were separated from each other, put with the inside face onto the cap rim and the other part stored) and the chamber closed with the cut tube piece. The dialysis cells were put into the beaker filled with 250 mL 1X SDB (2 M NaCl) and shaked in a way that the liquid came into contact with the membrane. All solutions were kept at 4 °C all the time. It was stirred for 1 h at about 60 rpm. After 1 h 250 mL TE were added → 1 M NaCl. After 1 h 250 mL were discarded from the beaker and 250 mL TE were added → 500 mM NaCl. After 1 h all but 50 mL were discarded and 450 mL TE were added → 50 mM NaCl. Stirring was continued for at least 1 h. The dialysis cells were removed from the beaker and dried on top with a pipette tip. The samples were pipetted from the dialysis cells by perforating the membrane on the non-liquid side, then sucked up and transferred into a low binding tube. A gel was poured to check the purity and the best conditions. As a reference 100 ng free DNA and half of the amount of each nucleosome titration was loaded.

To pour the 5% native PAGE gel 0.5X TBE, 1.875 mL 40% acrylamide and 750 µL 10X TBE were added into a 50 mL falcon tube. It was filled up to 15 mL with water, mixed and 150 µL 10% APS and 7.5 µL TEMED were added. It was mixed again, the gel poured into the cassette, the comb inserted and polymerized for about 40 min. After polymerization the chamber was rinsed to remove all SDS, the comb removed and the chambers washed with Millipore water. The gel was placed into the chamber and filled up with 0.5X TBE. For sample preparation, half of the nucleosome amount was mixed with 2 µL glycerol (cut tip end) and the chambers loaded. A 100 ng DNA sample as reference was also loaded. The gel was run at 90 V for 40 min. DNA bands in the gels were visualized using post-staining by allowing the gel to float in 100 mL 0.5X TBE and 10 µL SYBR Safe DNA Gel Stain (10000X) for 30 min. The bands were visualized with Chemidoc instruments.

For large scale assembly a reaction volume of 50 µL was used.

Week 2, group A2 - Analysis of the New DNA Binder by Circular Dichroism (CD):

CD measurements

References: *Curr. Protein Pept. Sci.* **2000**, *1*, 349.; *Biochemistry*, **1999**, *38*, 2143.; *Bioconjugate Chem.* **2015**, *26*, 2054.

Questions: What does CD stands for? How does a CD measurement works? What do we use CD measurements for? Why do we get a CD signal, although or molecule is not chiral? What is an ICD?

How the CD spectrometer was turned on and off:

Turn on:

- the nitrogen flow was turned on
- the nitrogen monitor (Afristo) was turned on and the gas flow checked (at least >2.5 L/min; best 5-10 L/min)
- the computer and screen were turned on
- the water bath (Haake WKL 26) and peltier controller (CDF426) were turned on
- the CD-spectrometer JASCO J810s was turned on
- the software SpectraManager was started: spectrum measurement was chosen from the list on the right
- It was waited for at least 20 min before the first measurement, to let the lamp heat up and stabilize
- it was proceeded by clicking measurement – accessory – Temperature: Jasco Peltier Controller – ok
- it was proceeded by clicking control – accessory – put the desired temperature – apply – close
- it was proceeded by clicking measurement – parameters – put the desired settings

Turn off:

- all data were saved (also as.txt file) and downloaded onto an USB-stick
- the software was closed and in the Spectra Manager “Instrument STOP” was chosen
- the JASCO J810s, water bath and peltier controller were shut down
- the nitrogen gas was turned off
- the nitrogen monitor was turned off
- the computer and the screen was shut down

How concentrations of a stock solution were determined:

A little bit of the polyamide hairpin sample was dissolved in 100 µL ddH_2O. It was vortexed well and all centrifuged down.

On the photometer, a 500 µL absorbance cuvette was filled with 500 µL of ddH_2O and a blank measured. 3 µL of the stock was added, well mixed and the absorbance at the wavelength given measured. Three data point between an absorbance between 0.3 and 1.0 were recorded. The solution was kept from the cuvette and from the stock and freeze dried on the lysophylizer after finishing with the measurements. The concentration of the stock was calculated with the equation:[10]

$$c(stock) = \frac{A}{\varepsilon} * \frac{V_{total}}{V_{sample}}$$

It was used:[11] ε_{310nm}(unmodified polyamide hairpin) in ddH_2O = 69200 $M^{-1}cm^{-1}$

How CD measurements were performed:

The following settings were used: 20°C, 0.2 cm pathlength, start: 380 nm, end: 220 nm, scanning speed 100 nm/min, response 0.25 s, data pitch 1 nm, accumulations 3, bandwidth 2 nm, sensitivity: high, scanning mode: continuous.

To have a 10 µM DNA solution in the end, it was calculated how much of the DNA stock was needed to get this final concentration in a volume of 500 µL. The 0.2 cm pathlength CD cuvette was filled with (500 µL – V (DNA stock)) 20 mM NaH_2PO_4 100 mM NaCl pH 7.4, the cuvette placed in the spectrometer, 5 min waited and the blank measured. The calculated DNA amount was added, carefully mixed while avoiding to produce bubbles in the cuvette, 3 min waited and measured. Increasing amounts (250 µM) of polyamide hairpin stock were added, carefully mixed while avoiding to produce bubbles in the cuvette, 3 min waited and measured. It was continued until the recorded spectra reached a saturation.

The data was exported from the CD-spectrometer and plotted in θ x 10^{-4} [deg/M*cm] versus the wavelength. The maxima of the ICD signal was plotted against the concentration of polyamide hairpin and the K_D calculated by using the program DynaFit.[12]

Week 2, group B2 - Analysis of the New DNA Binder by Fluorescence Intercalation Displacement (FID):

References: *Org. Lett.* **2010**, *12,* 216.*; Acc. Chem. Res.* **2004**, *37*, 61-69.

Questions: What is fluorescence? Why do we need two different types of cuvettes when we measure absorbance and fluorescence? What is a stokes shift? What is an absorbance, emission and excitation spectra? Why do we expect a change of fluorescence when we increase the concentration of DNA in our fluorophore stock? Why do we expect a decrease in fluorescence when we add our polyamide hairpin to the mixture?

How concentrations of a stock solution were determined:

It was followed the protocols of the CD measurements.

How the spectrometer settings were set:

To use the fluorimeter, first the computer was turned on, then the fluorimeter and the water bath. When all sounds of the fluorimeter were finished, the program was started and then the lamps turned on by using the program. It was waited for at least 20 min before the first measurement to let the lamp stabilize. Then only the emission lamp was turned on and auto scale pressed. Then only the excitation lamp was turned on and auto scale pressed. In the end, only the emission lamp was turned on. For measurements it was proceeded by clicking on file – properties and the setting typed in. Ok was pressed, the measurement started and the results saved. Then, it was continued with the next measurement.

How the polyamide hairpin titrations were performed:

Measurements were performed in a volume of 1 ml at a concentration of 6 µM thiazole orange and 1 µM DNA duplex with the right target sequence in 20 mM NaH_2PO_4 100 mM NaCl pH 7.4 with increasing amounts of polyamide hairpin.

The following settings were used: 20°C, excitation wavelength: 490 nm, excitation slidth: 3 nm, emission wavelength: 510 - 700 nm, emission slidth: 3 nm, scanning speed 500 nm/min, response 0.2 s, sensitivity: medium, data pitch: 1 nm.

To have a 6 µM thiazole orange and 1 µM DNA hairpin solution in the end, it was calculated how much of the stocks were need, to achieve this final concentration in a volume of 1 mL. The cuvette was filled with (1 mL – V (DNA stock) – V (thiazole orange stock)) 20 mM NaH_2PO_4 100 mM NaCl pH 7.4 buffer, the

cuvette placed into the spectrometer, 5 min waited and the blank measured. The calculated thiazole orange amount was added, carefully mixed while avoiding to produce bubbles in the cuvette, 1 min waited and measured. The calculated DNA amount was added, carefully mixed while avoiding to produce bubbles in the cuvette, 3 min waited and measured. Increasing amounts (250 µM and 1 mM) of polyamide hairpin stock were added, carefully mixed while avoiding to produce bubbles in the cuvette, 3 min waited and measured. It was continued until the recorded spectra were nearly absent.

To plot the fluorescence intensity versus the wavelength the data from the spectrometer was exported. The maxima of the fluorescence signal was plotted against the concentration of polyamide hairpin and the K_D calculated by using the program DynaFit.[12]

Week 2, group C2 - Analysis of the New DNA binder by Footprinting Combined with Capillary Electrophoresis (CE):

For this methodology only references, questions and the procedure and data of the final sample preparation are given due to non-published data.

References: *ChemBioChem* **2018**, *19*, 664-668.*; Org. Biol. Chem.* **2019**, *17*, 1827-1833.

Questions: What is capillary electrophoresis? On which methods is it based on? Which are the most common applications? How can a nucleosome dissemble? Why do we use different reagents for CE of free DNA and nucleosomes? Can potential minor groove binders bind at any position of the nucleosome? How does the common footprinting methodology works? What are advantages and disadvantages?

How concentrations of a stock solution were determined:

It was followed the protocols of the CD measurements.

Sample preparation:

The freeze-dried DNA amount was dissolved in 12 µL Formamid and 0.5 µL size standard (550 BTO, Biotype).

The CE samples were run on an *ABI PRISM 310* genetic analyzer by *Applied Biosystems*. All samples were separated on denaturing POP-4 polymer (*Applied Biosystems*) in a capillary with a length of 47 cm (36 cm well-to-read) and a diameter of 50 µm. After an electrokinetical injection of 5 s at 15 kV the samples were run for 28 min at 60 °C and 15 kV.

QUESTIONS TO START THE PROPOSAL

Before starting with the Pomodoro-technique, the students filled in a questionnaire to guide their own ideas and facilitate initial introduction among students. The questions in the questionnaire were:[13]

- What are your research interests?
- What are the problems in your interested fields? Why are they important?
- With what sources of data can you validate the importance of the proposed project?
- How is the existing knowledge inadequate?
- Why are your ideas better?
- What makes your project new / unique / different?
- What will it contribute and who will benefit from it?

POSTER TEMPLATE

For the proposal poster, the students were supplied with the poster template below as a guide. They were free to choose the template or use other approaches to present their idea.

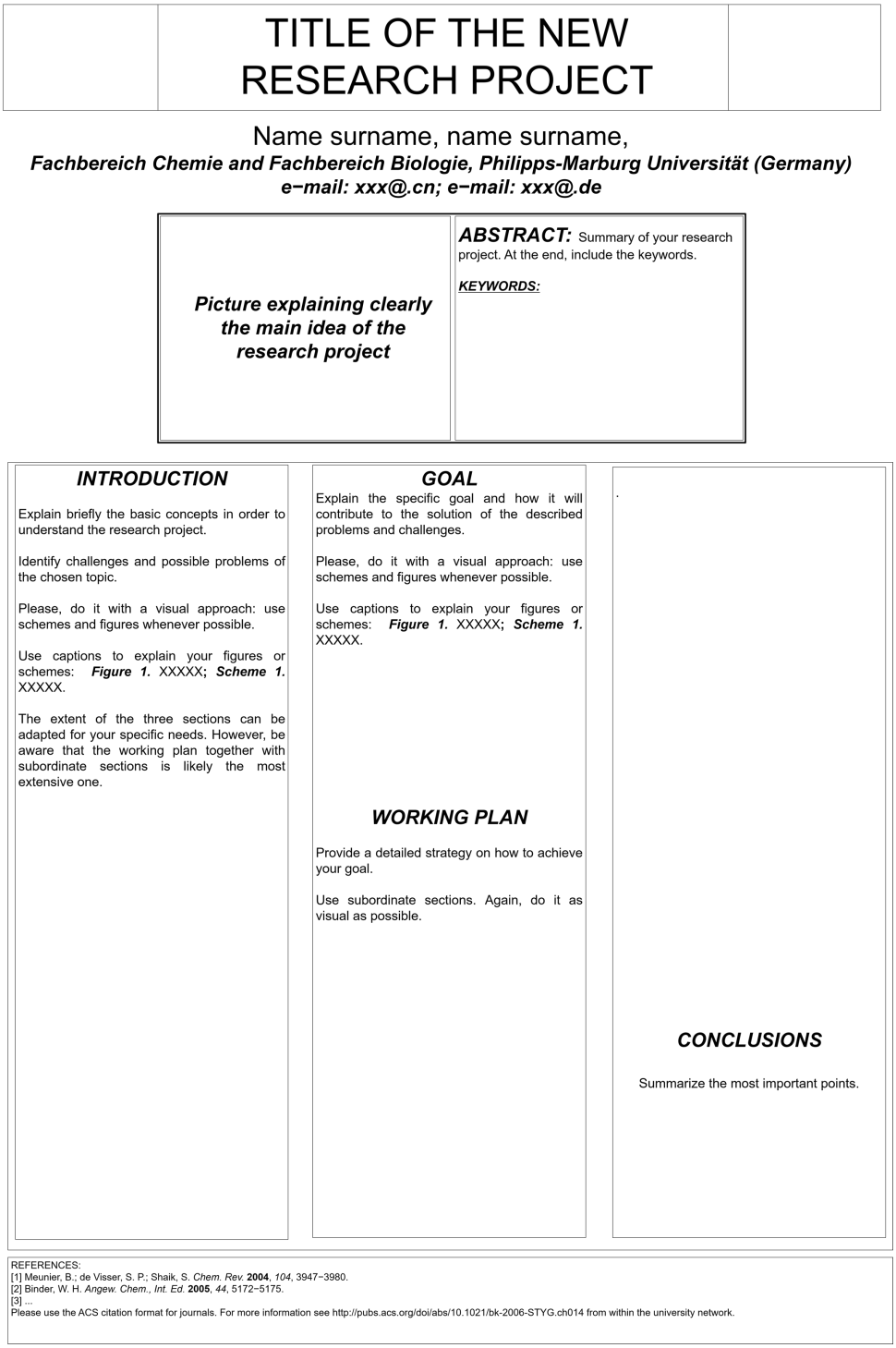


Figure S1: Poster template, which was provided for the students.

EVALUATION CRITERIA FOR DISCOVERY-BASED RESEARCH LAB

To assess the outcome of the students, different criteria for each experimental technique were used (below).

Synthesis:

All synthesized monomer precursors were literature described.[3] Therefore, the access of the molecules, yields and characterization by ^{1}H and ^{13}C NMR spectroscopy and CI or ESI mass spectrometry can be easily evaluated.

The synthesized polyamide hairpins were novel. To judge the success of the synthesis HPLC-MS analysis was performed. The number of peaks revealed how many truncations and other side products were formed and mass analysis helped to assign the chromatograms. In the ideal case, the HPLC-chromatogram would show a single peak and a mass spectra containing only masses, which belong to the product polyamide.

PCR and nucleosome assembly

The success of the PCR and nucleosome assembly as well as their purity was tested by native polyacrylamide gel electrophoresis (PAGE). Supplying free DNA and nucleosome reference samples and/or the addition of DNA ladder validated the size of the bands in the gel and allowed the assessment of the results.

CD measurements

To assess the results of the CD measurements we compared the observed signals with the literature reported of known similar compounds. After addition of dsDNA hairpin to the buffer the typical bands for B-DNA: minimum at 250 nm and a maximum at 280 nm were expected.[9] Addition of increasing amounts of polyamide resulted in an induced CD signal at the absorbance range of the polyamide (300-360 nm).[14] Absence of contamination by the students could be evaluated by initial measurements of the buffer in which no distinct signal was expected. Overlay of the buffer- and DNA-spectra with previous ones, performed by the instructors helped to assess the results.

Final calculation of the binding constant allowed the students and the instructors to compare their results to known literature values in the nM-region[14] and judge their measurements.

FID measurements

FID measurements were performed with 6-TramTO-3.[15] In the first cohort we used this fluorescent dye, which was developed in our own laboratory. After finding out with the help of the students that it was not a suitable dye for FID measurements, we changed to thiazole orange for the second cohort. To assess the results of the FID measurements it was checked, if the observed signals align with the expectations

in comparison to known compounds. Fluorescence measurements of the buffer alone and of the buffer and the dye gave the expected weak fluorescence intensity signals. After addition of the DNA to the dye solution, the typical strong fluorescence signals at 645 nm for 6-TramTO-3[15] and 530 nm for TO[16,17] was anticipated. Addition of subsequent amounts of polyamide resulted in a decreasing fluorescence signal.[16,17] Absence of contamination by the students could be evaluated by initial measurements of the buffer in which no distinct signal was expected.

Final calculation of the binding constant allowed the students and the instructors to compare their results to known literature values in the nM-region[16] and judge their measurements.

General evaluation

The discovery-based research lab was evaluated following the criteria given below:

- Did the students achieve the synthesis/PCR and nucleosome assembly/CD measurements/FID measurements?
- How do the students work in the lab?
- Does the analysis data of the synthesis fit the reported ones from the literature?
- How are the yields and the purity?
- Do the measurements look as expected?
- Do the calculated data fit the region from the literature?
- Are the students able to evaluate and interpret their data?

EVALUTATION CRITERIA FOR THE SCIENTIFIC COMMUNICATION

The scientific article and the research proposal poster, including the presentation were evaluated following the given guidelines:

Scientific article:

- Was the communication prepared using the journal template?
- How is the general / first impression?
- Does the communication follow the general structure and addresses each content appropriately?
    - Title: does the title suits to the content?
    - Abstract: appropriated structured and summarizing the relevant content?
    - Introduction: scope of background info? Connection between precedents and new results?
    - Is there a scientific gap to highlight the importance of the article?
    - Discussion: well discussed results? Importance of bigger picture?
    - Methods: clear and complete?
    - References: complete and following the right style?
- What data were used?
- Does it have scientific rigor?
- Is the used language clear and understandable?
- Are the figures coherent with the data?

Research proposal poster:

- Is it well structured?
- How is the general / first impression?
- Does the topic cover the field of epigenetics?
- Is it a novel project?
- Is the timeline and methodology feasible?
- Is it visual and understandable?
- How do the students present their poster:
    - Did they understand the topic and the precedents?
    - Do they present their poster well?
    - Are all students taking part equally?
    - Are they able to answer (defence) questions?

STUDENT'S OUTCOME

In this section, examples of student's outcomes for the lab course, the scientific article and the poster are given. Tables which describe the criteria, which were used to evaluate and rate the outcomes, are further supplied.

OUTCOMES OF THE DISCOVERY-BASED RESEARCH LAB

Synthesis:

Below, students' results of the ^{1}H-NMR, ^{13}C-NMR and HRMS are noted and exemplary a figure of the corresponding ^{1}H-NMR spectra is shown.

Py-CCl_3:

^{1}H-NMR (300 MHz, DMSO-d_6, δ): 7.46 - 7.42 (m, 2H, C*H*-1 and C*H*-3), 6.30 (dd, 1H, J = 4.3 Hz, J = 2.5 Hz, C*H*-2), 3.91 (s, 3H, C*H*-4).

^{13}C-NMR (75 MHz, DMSO-d_6, δ): 171.8 (C=O), 135.3 (CH), 123.6 (CH), 120.7 (C_q), 109.1 (CH), 96.0 (CCl_3), 36.2 (CH_3).

HRMS-CI$^+$ (m/z): $[M+H]^+$ calcd for $C_7H_6Cl_3NOH$, 225.95932; found, 225.95978.

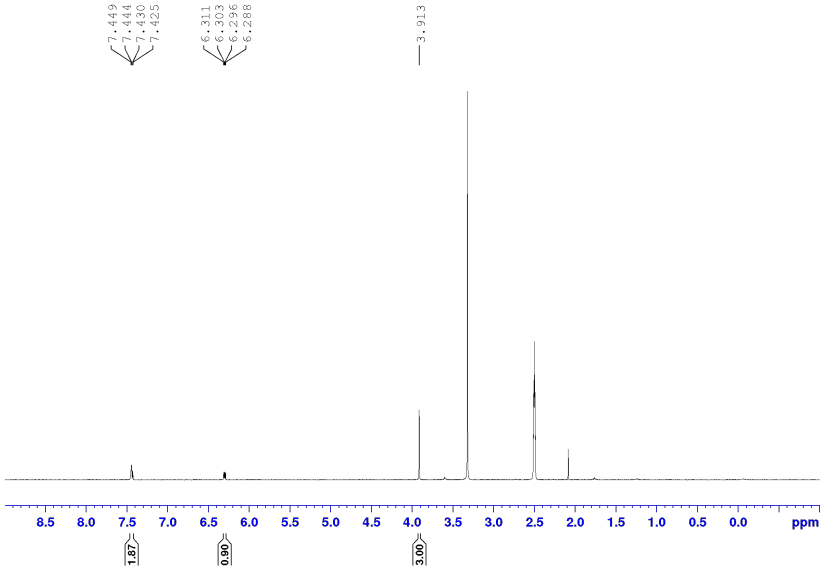


Figure S3. ^{1}H-NMR spectra of Py-CCl_3.

NO_2-Py-CCl_3:

^{1}H-NMR (300 MHz, DMSO-d_6, δ): 8.56 (d, 1H, J = 1.6 Hz, C*H*-2), 7.80 (d, 1H, J = 1.6 Hz, C*H*-1), 4.00 (s, 3H, C*H*-3).

^{13}C-NMR (75 MHz, DMSO-d_6, δ): 172.8 (C=O), 134.2 (C_q), 132.5 (CH), 120.6 (C_q), 116.3 (CH), 94.5 (CCl_3), 50.0 (CH_3).

HRMS-CI$^+$ (m/z): $[M+H]^+$ calcd for $C_7H_5Cl_3N_2O_3H$, 270.94440; found, 270.94291.

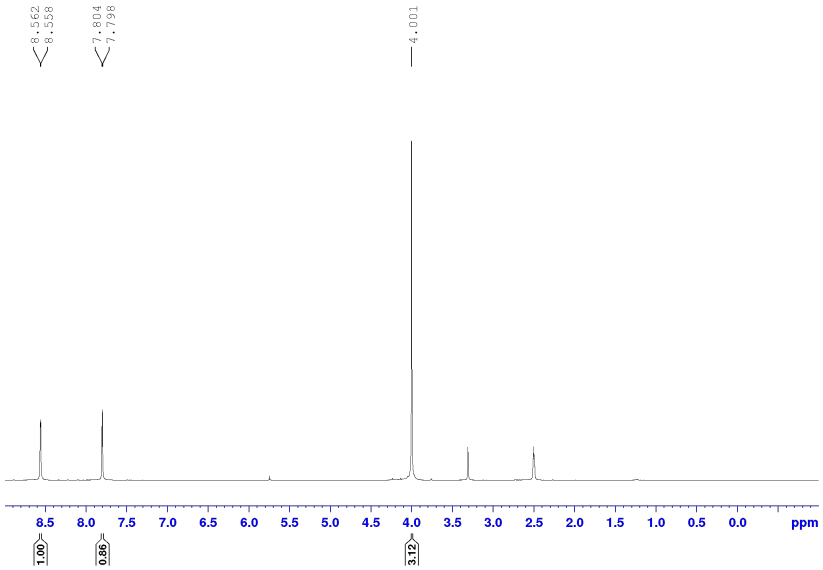


Figure S4. ^{1}H-NMR spectra of NO_2-Py-CCl_3.

NO_2-Py-OMe:

^{1}H-NMR (300 MHz, DMSO-*d*$_6$, δ): 8.27 (d, 1H, *J* = 2.0 Hz, C*H*-2), 7.30 (d, 1H, *J* = 2.0 Hz, C*H*-1), 3.92 (s, 3H, C*H*-3), 3.80 (s, 3H, C*H*-4).

^{13}C-NMR (75 MHz, DMSO-*d*$_6$, δ): 159.8 (C=O), 134.2 (C_q), 129.4 (CH), 122.6 (C_q), 111.5 (CH), 51.7 (CH_3), 37.4 (CH_3).

HRMS-ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_7H_8N_2O_4H$, 185.05623; found, 185.05665.

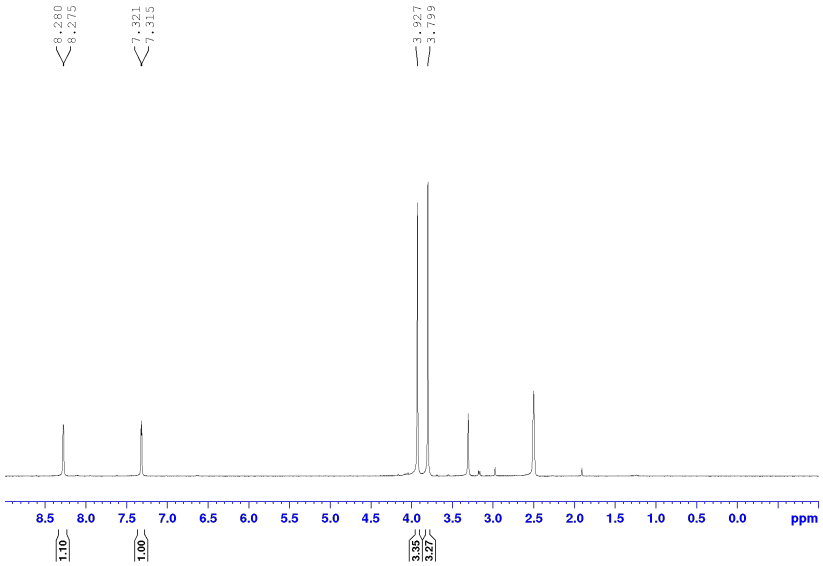


Figure S5. ^{1}H-NMR spectra of NO_2-Py-OMe.

Im-CCl_3:

^{1}H-NMR (300 MHz, CD_3OD, δ): 7.31 (s, 1H, C*H*-2), 7.08 (s, 1H, C*H*-1), 4.01 (s, 3H, CH_3-3).

^{13}C-NMR (75 MHz, CD_3OD, δ): 160.3 (C=O), 137.8 (C_q), 129.4 (CH), 128.1 (CH), 119.5 (CCl_3), 36.2 (CH_3).

HRMS-CI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_6H_5Cl_3N_2O_1H$, 226.95457; found, 226.95069.

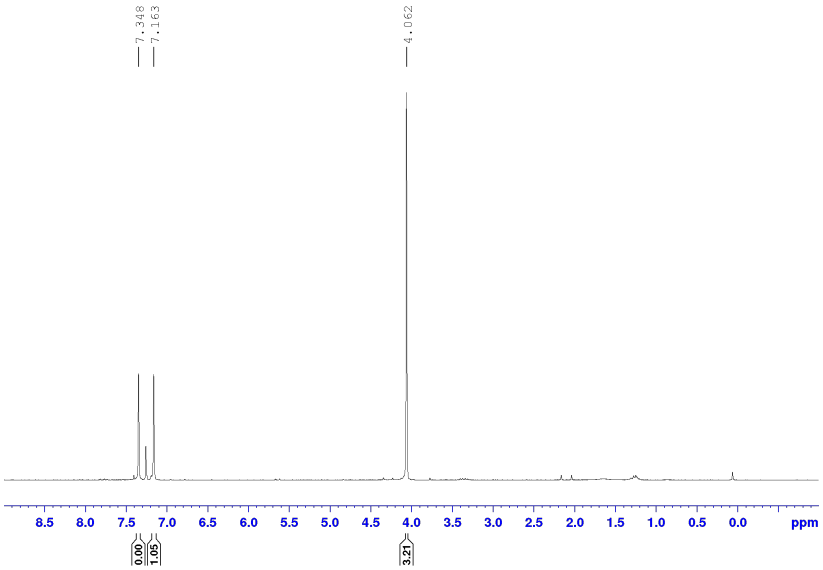


Figure S6. ^{1}H-NMR spectra of Im-CCl_3.

NO_2-Im-CCl_3:

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.95 (s, 1H, C*H*-1), 4.16 (s, 3H, CH_3-2).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 173.0 (C=O), 146.0 (C_q), 133.8 (C_q), 126.1 (CH), 93.7 (CCl_3), 38.4 (CH_3).

HRMS-CI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_6H_4Cl_3N_3O_3H$, 271.93965; found, 271.94021.

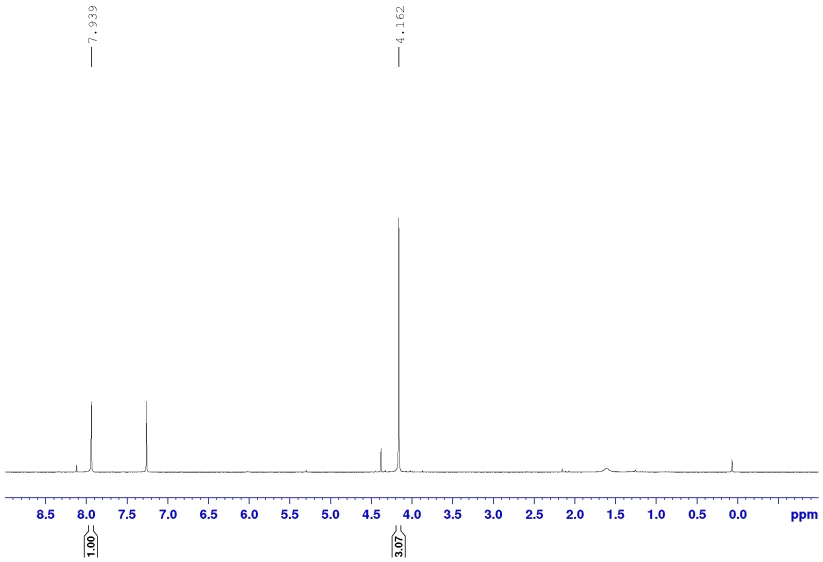


Figure S7. ^{1}H-NMR spectra of NO_2-Im-CCl_3.

NO_2-Im-OMe:

^{1}H-NMR (300 MHz, CD_3OD, δ): 8.29 (s, 1H, C*H*-1), 4.08 (s, 3H, CH_3-2), 3.96 (s, 3H, CH_3-3).

^{13}C-NMR (75 MHz, CD_3OD, δ): 159.6 (C=O), 142.9 (C_q), 136.6 (C_q), 126.9 (CH), 53.2 (CH_3), 37.5 (CH_3).

HRMS-ESI$^+$ (*m/z*): $[M+Na]^+$ calcd for $C_6H_7N_3O_4Na$, 208.0329; found, 208.0328.

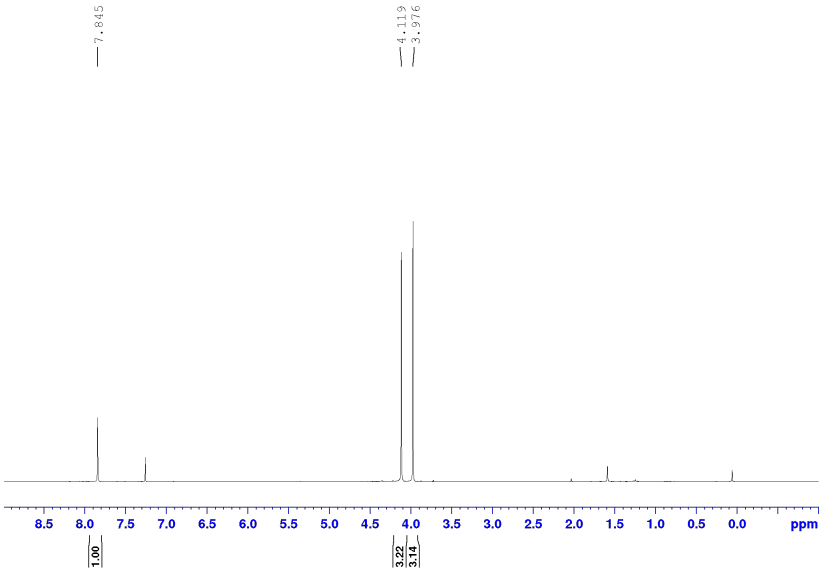


Figure S8. ^{1}H-NMR spectra of NO_2-Im-OMe.

Polyamide Hairpin Synthesis:

Figure S9 shows an exemplary HPLC-chromatogram of the intermediate nucleosome polyamide 1 after 4 couplings from the students' progress report.

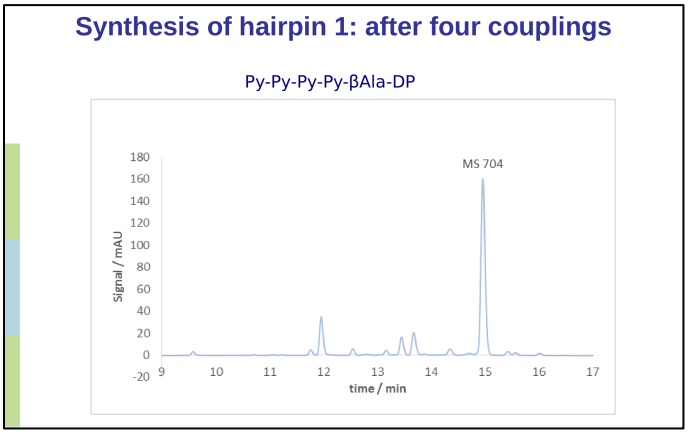


Figure S9. HPLC chromatogram of a test cleavage of intermediate nucleosome polyamide 1 after 4 couplings with marked mass, which was found in the product peak. The figure represents a slide of the students' progress report seminar.

Figure S10 and S11 shows the final chromatograms after purification of both nucleosome polyamides. Purification was performed on a VARIAN ProStar system with a preparative Juptier 10 u C18 300 Å column (10 µm, 250 x 10 mm; Phenomenex) using a flow rate of 8 mL/min at 40 °C. Analysis was performed on an Agilent 1200 Series HPLC-System (Agilent Technologies) with an eclipse XDB-C18 column (5 µm, 4.6 x 150 mm, Agilent). Milli-Q water (A) and MeCN (B) were employed as eluents with an addition of 0.05% of TFA for A and 0.03% for B. The flow rate was 1.0 mL/min using an isocratic regime of 5% B during the first five minutes, for column equilibration, followed by the linear gradient 5% to 95% B in 30 min at 55 °C.

Nucleosome Polyamide 1:

R_t = 16.1 min.

MS *m/z* = 1279.4 $[M+H]^+$, 640.4 $[M+2H]^{2+}$.

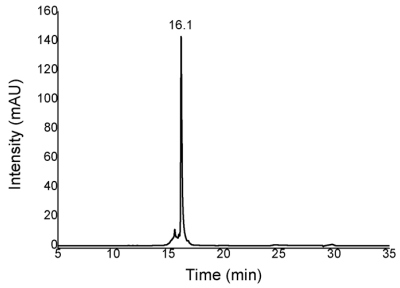


Figure S10. HPLC chromatogram of the purified nucleosome polyamide 1. Gradient 5-95% B.

Nucleosome Polyamide 2:

R_t = 16.1 min.

MS *m/z* = 1279.4 $[M+H]^+$, 640.4 $[M+2H]^{2+}$.

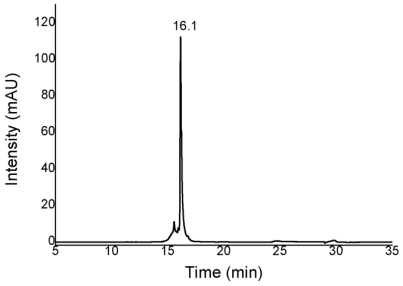


Figure S11. HPLC chromatogram of the purified nucleosome polyamide 2. Gradient 5-95% B.

PCR and nucleosome assembly

In the figure below, lane-1 shows the free DNA-W601 from the PCR. Lane 2-8 are the results of nucleosome test assemblies with increasing amounts of histone octamers while keeping the DNA amount constant.

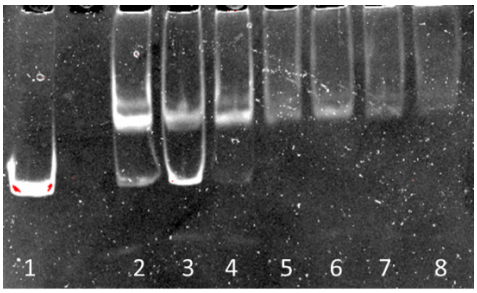


Figure S12. Native PAGE analysis of free DNA-W601 in lane-1 and nucleosome test assemblies in lane 2-8 with increasing amounts of histone octamers.

CD-measurements

Figure S13 shows an exemplary CD titration with polyamide **1** taken from a student's communication.

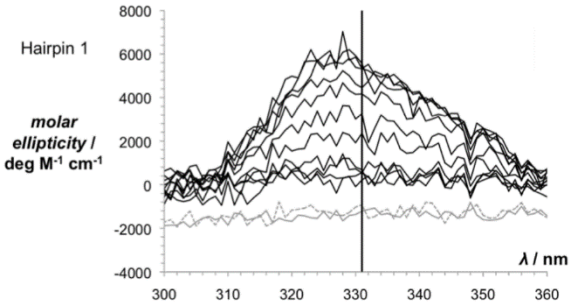


Figure S13. Exemplary CD titration of DNA with polyamide hairpin **1**. CD signal increase upon addition of more polyamide.

FID-measurements

Figure S14 presents an example of a student's result of the FID titration of polyamide **1** with 6-TramTO-3.

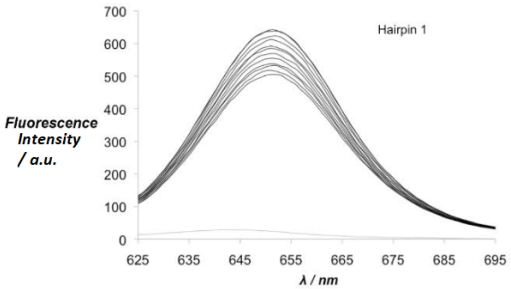


Figure S14. Exemplary FID titration of 6-TramTO-3 and DNA. Subsequent addition of polyamide resulted in a decrease of fluorescence signal intensity.

Synthesis and characterization of polyamide hairpins as small DNA binder.

Name Surname [a], Benedikt Heinrich [b] and Jun.-Prof. Dr. Olalla Vázquez*[b]

Abstract: To study molecular processes, which are involved in changing expression patterns of DNA, have become a central issue in epigenetics. Repression of overexpressed histone deacetylases (HDAC) is one of the powerful tools against several cancer types. Polyamide hairpins have high potential as external DNA binders and are therefore potential anti-cancer drugs. Nowadays only a few numbers of DNA binding small molecules exist. Here, we show the immense potential of polyamide hairpins and provide a unique mechanism to establish inducible binding. We synthesized two new hairpins and determined the dissociation and sequence specificity to free DNA as well as nucleosome core particle (NCP). Our results demonstrate reliable binding of polyamide hairpins in order to minimize access to specific DNA sequences. Future studies will be directed in access hairpin controlled gene expression *in vitro*.

DNA is the genetic basis for nearly all organisms except phages. In humans, the DNA double helix is wrapped around histone octamers to form the nucleosome. The unit of the nucleosome is the nucleosome core particle (NCP) what can contain several modifications as histone sequence variants, posttranslational (PTM) modifications, mutations and more [1].
Histone deacetylases (HDAC) regulate posttranslational modifications (PTM) of histone tails and therefore have an impact in DNA compaction. Due to less access of transcription machineries to the DNA with higher density, HDAC have consequently an impact in transcription. But HDACs have multiple roles. Some cause deacetylation of amino-terminal lysine residue at the tail of histone H3 and therefore promote tighter compaction of positively charged histones and negatively charged DNA. Beside this transcription repression they are also responsible for deacetylation of several non-histone proteins as sigma factors, signal modulators and play a major role in PTMs [2]. Nevertheless HDACs were found to be overexpressed in a broad range of cancer types [3]. For that reason find therapeutic targets for controlling aberrant HDAC expression remain a significant challenge for the next decades.

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Hairpins or so-called Dervan hairpins are polyamides consistent of *N*-methylpyrrole (py) and *N*-methylimidazole (im). These hairpins are used to target specific DNA sequence trough side-by-side recognition of the minor groove of DNA.
Dervan hairpins are used hence as synthetic ligands for external DNA binding. Consequently Dervan hairpins demonstrate their practical application against DNA deacetylation and further more as anti-tumor drug [4].
For that reason we firstly synthesized two different hairpins. Accordingly, we characterize our newly synthesized compounds in circular dichroism (CD) and fluorescence intercalation displacement (FID), which is a highly competitive method to determine binding affinity. Further we analyzed the sequence specificity. To observe the sequence specificity was used a modified footprint method, which exclude the work with ^{32}P or ^{33}P radioactive labelled probes and therefore provide a facilitated laboratory use [5].

Initially were synthesized two different hairpins, which varies in the arrangement of py and im (Fig. 1 (A-B)). According to the side-by-side recognition of im and py to the DNA double helix sequence specificity is generated for each hairpin individual (Fig. 1 (C)).

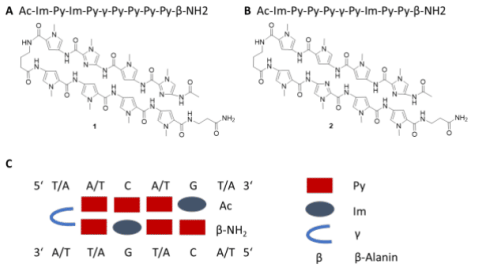

Figure 1. Synthesized hairpins. Hairpin **1** (A) and hairpin **2** (B) differ in amid arrangement and therefore in sequence specificity. (C) Shows sequence specificity of hairpin **2** and pairing rules. A: Adenine; T: Thymine; G: Guanine; C: Cytosine; Ac: Acetyl group.

Subsequent to characterize the newly synthesized hairpins were made some CD spectroscopy measurements. In order to induce chirality were used a DNA hairpin mixture and the molar ellipticity were determined from wavelengths 220 nm to 380 nm for hairpin **1** (Fig. 2).

As we can see from figure 2, hairpin **1** bound to the DNA and therefore an increasing molar ellipticity is observed around 325 nm. The general ability of hairpin **1** to bind to DNA could be hereby observed. As also indicated, that the

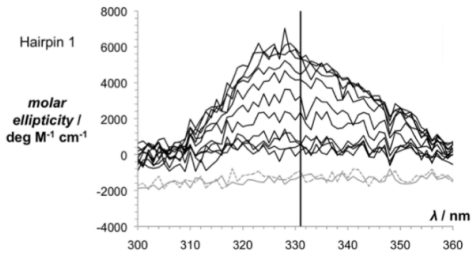

Figure 2. Induced CD spectroscopy of hairpin **1**. In grey are measurements without addition of DNA or hairpin (discontinuous) and the second one without hairpin and with the addition of DNA (grey). In black are added increasing hairpin concentrations to DNA. The hairpin concentrations varies from 0,7 µM to 33 µM.

increasing ellipticity is coupled to an increasing amount of the hairpin. The lowest hairpin concentration causes the lowest shift as well as the highest hairpin concentration causes the highest shift as visible in figure 3. To further characterize the binding intensity of hairpin **1** the dissociation constant (K_D) was calculated (Fig. 3).

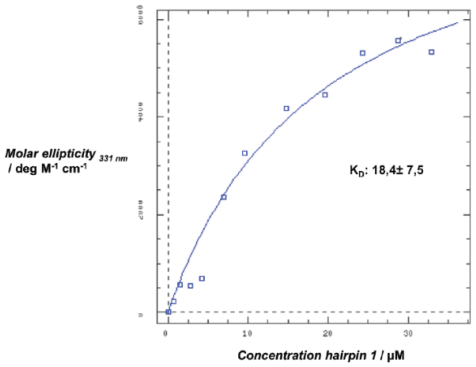

Figure 3. Molar ellipticity at 331 nm wavelength with various concentrations of hairpin **1** from 0,7 µM to 33 µM . K_D of 18,4 µM ± 7,5 µM was determined with DynaFit.

The K_D values correlate to already published constants that are in a range between 1,1±0,3 µM and 5,9±0,7 nM for comparable polyamides [6]. Nevertheless the K_D value for hairpin **1** could be determined, according to the high standard deviation it is indicated that the measurements were less than ideal (Fig. 3).

To extend the characterization of hairpin **1** further fluorescence spectroscopy were performed to analyze the binding. It was chosen a competitive test. To test the binding affinity was used 6-TramTO-3 [7] cyanine dye triazole to label the DNA via fluorescence around 650 nm wavelengths. The dye is only detectable when intercalated into DNA. DNA and dye itself have no fluorescence (Fig. 4). With increasing concentrations of DNA binder (hairpin **1**) the dye should be displaced and the fluorescence decreasing.

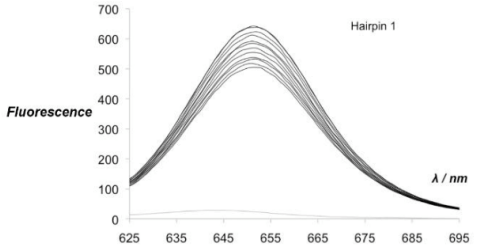

Figure 4. Fluorescence displacement with 6-TramTO-3 cyanine dye triazole. Detection of the binding of DNA to hairpin **1**. In grey (discontinuous) was only added DNA; in grey was only added 6-TramTO-3 cyanine dye triazole. In black were used increasing amounts of hairpin **1** from 1 µL to 10 µL in addition to DNA and 6-TramTO-3 cyanine dye triazole. Stock concentration hairpin 1 was 216 µM. Fluorescence intensities were followed from 625 nm to 700 nm.

As can be seen from figure 4 that hairpin **1** can successfully displace, with increasing concentration, the cyanine dye. According to previous CD spectroscopy measurements, were these results expected. It is confirmed that hairpin **1** could serve as small molecule binder. Even the conditions were not that precise and the measurements should be repeated.

After we showed that our hairpin bind DNA, it is important to prove the sequence specificity of hairpins in order to ensure an accurate gene repression. Therefore was used a footprint method. The method was modified. Instead of radioactive labeling was used 6-carboxyfluorescein (6-FAM). Free DNA (W601) and DNA (W601) coupled to histone octamers (NCP) were tested. According to amid arrangement (Fig. 1 (C)) of hairpin **2** were one potential binding site predicted (Fig. 6, red entangled). Footprint method is based on DNAse fragmentation. Potential binders prevent specific sequence from disruption. Capillary electrophoresis generates afterwards a specific fragment pattern where each peak contains fragments with

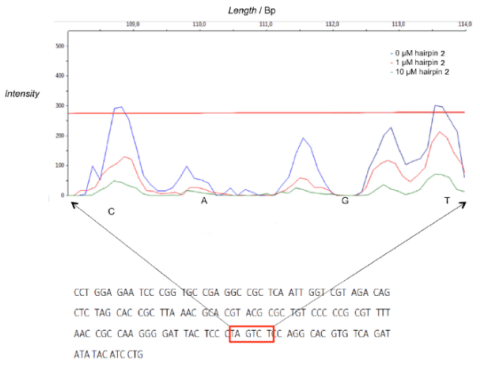

Figure 5. Detail of the predicted binder site. Top: Intensity of fragments with lengths between 108 bp and 114 bp. Hairpin **2** was used in two concentrations (0 µM and 10 µM) with free DNA (50 µM). Bottom: DNA sequence of DNA W601. Red entangled: Predicted binder site. It was used 4 mU DNase I.

specific base pair (bp) length. The higher the intensity of the peaks the more fragments are developed. Fragment pattern of the potential binding site is shown in figure 5.
At the predicted binding site were the results remarkable good. It is clearly visible that with increasing hairpin **2** concentrations the intensity of these fragments is decreasing. Because of the binding of hairpin **2** is the access for the DNase I limited. Consequently the fewer fragments were cut at this position the less fragments arise with this specific length.

It is suggested that hairpins can bind the minor groove of DNA. When DNA is associated to histone octamers for compaction seven minor grooves are potential binding sites. To characterize the hairpin further, it is most important to determine the sequence specificity to the NCP. The potential binding site was again investigated trough footprint method (Fig. 6).

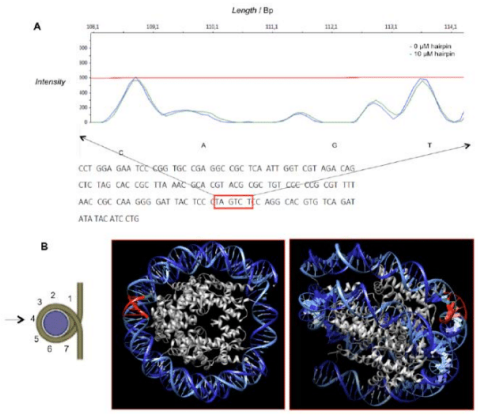

Figure 6. Potential binding site of hairpin **2** to DNA of NCP. It was used 0 µM and 10 µM hairpin **2** and 100 µM NCP. DNAse I concentration was used 400 U (A). (B) Predicted binding site is located in the 4th minor groove of DNA (indicated in red). Blue: DNA double helix; Grey: Histone octamers [8] images were modulated with the help of UCSF chimera.

For the predicted binding site is no difference with or without hairpin **2** visible in fragment pattern intensity. In this case we cannot confirm the binding affinity of hairpin **2** to the NCP. More or less have to be checked further the potential binding site in context of the structural assembly. It might that there are steric hindrance problems due to interactions with the NCP that restrict the binding ability. Nevertheless the binding site seems free of histone tails or other visible elements (Fig. 6 (B)). Despite this, the experiments should be repeated to come to a final conclusion. Although the binding was not obtained experimentally, it can be assumed to be possible. These dates are not representative.

In conclusion it could be proved the general ability of polyamide hairpins as small DNA binding molecules. Furthermore the new synthesized hairpin **1** could be characterized in binding to DNA via induced CD spectrometry and trough fluorescence displacement via fluorescence spectroscopy. Also for hairpin **2** the DNA binding was proven trough footprint. Reasonable results were also shown in sequence specificity of hairpin **2** to free DNA even when the binding to the NCP was not detectable. It is evident from the results the immense potential of polyamide hairpins as DNA binder. For investigations we also provide a modified footprint method to analyze specific binding sites, which ease the laboratory daily routine and showed a great application for fluorescence displacement.

As discussed previously are polyamides grateful tools to target specific sequences and interrupt the DNA-protein interaction as observed for DNase I. This might be transferred also to the interaction between transcription machinery and DNA. Finally it is possible to control the expression of specific DNA regions such as HDACs in order to limit the expression of these genes. Due to the nearly unlimited arrangement of the py and im monomers, this system offers a bright repertoire of targetable sequence. According to the pleiotropic role and relevance of HDAC in multiple completely different but essential cell processes, it remains a high risk of several unknown reaction in a potential in vivo application. This project opens a starting point for a new linage of polyamide modifications and couplings. As outlined in the introduction are next steps to determine the efficiency of gene repression in vitro. But the non-natural small molecules, which bind DNA specifically, are potentially dynamic tools in human medicine.

Experimental Section

Hairpin synthesis, purification and analysis

Hairpins were prepared by solid-phase synthesis using *N*-Fmoc protected intermediate steps. Concentration and isolation were done and determined by HPLC [9].

CD Spectrometry

For the CD measurements were used JASCO J810s CD spectrometer and Software SpectraManager. 5 µM DNA, 10 mM sodium phosphate 100 mM NaCl pH 7,5 were used to induce chirality. K_D was calculated through the program DynaFit. Spectra were measured from 220 nm to 380 nm.

Fluorescence displacement

1 µM cyanine dye 6-TramTO-3 and 6 µM DNA in 10 mM Tris 50 mM KCl pH 7,6 with increasing amount of hairpin were used. K_D were calculated using the program DynaFit. Fluorescence were measured using a fluorimeter. Excitation wavelengths were used 610 nm and emission wavelength 625 nm-750 nm.

Footprint experiments

To reconstitute NCP were used 1 µg W601 DNA and histone octamers prepared from chicken erythrocytes. For assembly were performed NaCl from 2M to 50 mM salt gradient dialyses. It was used 50 ng DNA (W601) and 100 ng NCP601 with different concentrations of hairpin **2**. For free DNA (W601) were used 4 mU DNase I and for NCP 400 U DNase I. Incubation time was 60s. To analyze the footprint pattern were used capillary electrophoresis.

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Conflict of interest statement. Non declared.

Keywords: Amides • DNA recognition• Drug design

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EXAMPLE OF A STUDENT'S RESEARCH PROPOSAL POSTER

Exploring the Substrate Scope of MOF Catalyst for p53 Acetylation and Validating the Influence of PHF20 on p53 Acetylation

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MOF-KAT

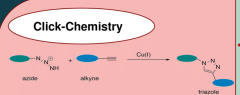
MOF is a lysine acetyltransferase which belongs to the MYST family. MOFs main function is depositing an acetylation to histone H4K16, but it also targets other histones as well as non histone proteins. It contributes to DNA damage and repair, cell survival and gene expression regulation. In humans it occurs in two different protein complexes, the MOF-NSL and the MOF-MSL complex. Only the MOF-NSL complex targets p53 K120.[8]

PHF20

PHF20 is a component of the MOF-NSL complex and regulates MOFs acetylating activity. The regulatory mechanism for H4K16 acetylation through MOF by PHF20 depends on the PHD subunit of PHF20 (shown below) binding to H3K4me. PHF20 subunit Tudor2 binds to p53 at K370me2 and K382me2, this interaction leads to stabilization and activation of p53. It may be that acetylation of p53 at lysine 120 done by MOF is also dependent on or influenced by PHF20.[2]

Recently PHF20 has been shown to regulate MOFs acetyltransferase activity acetylating H4K16. Since MOF also acetylates p53 which is an important tumour suppressor, the question arose whether p53 acetylation by MOF is also dependent on PHF20. Therefore we will perform in vitro and cell culture experiments to test this. Click chemistry can be used to detect acetylations. Different derivatives of acetyl-CoA are prepared which serve as substrate for acetylation. In a second step these acetylations can be tagged with fluorescence or purification tags. We use this approach to analyse the in vitro as well as the cell culture experiments via fluorescence (in vitro) and also to purify all acetylated products from the cell lysates. We also use western blot and LC-MS^n for analysis. In the in vitro assay several proteins are combined and analysed. For cell culture experiments we knock down PHF20 in one cell line and compare its acetylation level at p53 K120 with the acetylation levels acquired in the wild type.

Click-Chemistry



Click-chemistry is the generic term for a wide variety of highly reliable, powerful and selective reactions for rapid synthesis through heteroatom links (C-X-C).
Reactions that are click-chemistry have to accomplish a number of criteria. They have to be wide in scope and give very high yields. They should have only inoffensive by-products which must be removable without chromatically methods. A good stereospecificity and simple reaction conditions are necessary for click-chemistry as well as available starting materials and no or benign solvents (e.g. H_2O) and easy product isolation.[1].

In vitro Experiments:

Synthesis:
-Sodium acetate salts
-Coenzyme A derivatifs
-Azido-diazo-biotin
-H4 tail 1-19 aa

Different protein combinations with MOF

Acetylation detection with:
-Western blot
-LC-MS^n
-Fluorescence

Acetylation detection with:
-Western blot
-LC-MS^n
-Fluorescence

4 Weeks | 6 Weeks | 6 Weeks | 4 Weeks | 4 Weeks | 4 Weeks

Overexpression:
-p53 (full length and DNA binding domain)
-PHF20
-MOF

Celline preparation:
Expression of tagged p53 in H1792

Cell Culture:
Knock down of
MOF
PHF20
Each in prepared H1792 cells

Metabolic labeling of all cells

p53

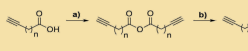
p53 is a tumour suppressor and a transcription factor. It is involved in cell cycle arrest, apoptosis, DNA damage response and metabolism. In the vast majority of tumour diseases p53 is mutated or deleted. This makes it a very attractive target for cancer treatment. Despite p53 being well characterized, there is still no general treatment applicable in p53 compromised tumours. So every new compound associated to p53 and influencing its function could harbour a new possibility to target p53 and result in a cancer treatment option.[3].

The first step the synthesis of all needed compounds which are:

1) Coenzyme A derivatives as substrates for the in vitro assay
2) Corresponding Sodiumacetate salts which are used in metabolic labeling
3) Azido-diazo-biotin is going to be the tag in the cell culture experiments to extract all acetylated compounds from the lysate

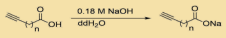
Synthesis of the Substrates

General synthesis of coenzyme A derivatives (time needed: ~ 5 h):



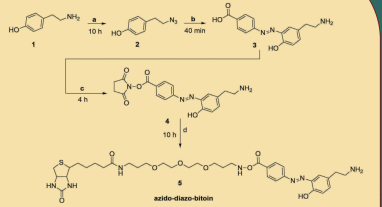
Scheme 1. General reaction scheme for the synthesis of the CoA-derivatives. Whole synthesis done under Ar-atmosphere
a) Formation of the anhydride: 2 eq carboxylic acid in anhydrous DCM N,N'-dicyclohexylcarbodiimid (DCC) added, stirred for 4 h at r.t.
b) dry crude product of **a)** solved in anhydrous DMF at 0°C, 0.2 eq coenzyme A hydrate **(CoA)** and 0.6 eq Et_3N added, stirred for 30 min at 0°C[4].

General synthesis of the sodium salts (time needed: ~ 1h):



Scheme 2. General reaction scheme for the synthesis of the sodium salt derivatives.1 eq of the carboxylic acid solved in ddH2O and NaOH added dropwise. Mixture filtered through membrane, frozen in $N_{(l)}$ and lyophilized to dryness[4].

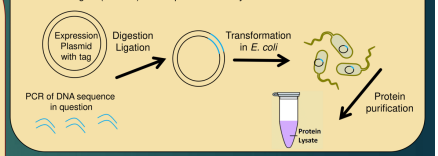
Synthesis of azido-diazo-biotin



Scheme 3. **Synthesis of azido-diazo-biotin (5)**: **a)** To an ice-cooled solution of NaN_3 (1.44 eq) in CH_3CN, slowly added Tf_2O (1.2 eq) and stirred for 2 h at 0°C. A solution of of tyramine (3.00 eq) in CH_3CN added and stirred for 8 h at 0°C. (lit.: 96%) **b)** $NaNO_{2(s)}$ added to ice-cooled suspension of 4-aminobenzoic acid in 6M HCL (40 mL), stirred at 0°C → reaction colour turned yellow. **(2)** (1 eq) dissolved in cooled THF at 0°C. K_2CO_3 added subsequently to reach pH=8. Diazonium salt solution added slowly to **(2)** at 0°C. pH kept around 8 by adding K_2CO_3. (lit.: 45-55%$_{\text{(over two steps)}}$) **c) (3)** (1 eq) dissolved in anhydrous THF, DCC (1.09 eq) and N-hydroxysuccinimide (1.09 eq) added under Ar-atmosphere. Stirred for 4 h at r.t., concentrated in vacuum, crude solid residue dissolved in chilled EtOAc, Urea filtered off. (lit.: 79%) **d)** Biotin-PEG-NH_2 (2 eq) added to solution of **(4)** in anhydrous DMF. (lit.: 80%)[4]

Protein over expression

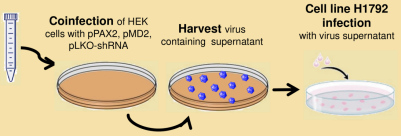
All proteins needed in the in vitro assay are expressed and purified from *E. coli*.
These are: p53; PHF20; MOF and only the DNA binding domain of p53 (DBD)
The H4 tail region (1-19 aa) can be purchased or synthesized.



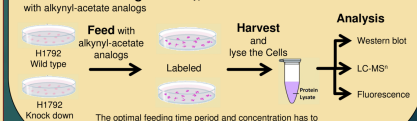
For the cell culture experiments our wild type cell line H1792 has to be modified at first, because we need it to express tagged p53 that we can purify p53 before analysing the acetylation with LC-MS^n. This is done with the same procedure as the knock down of PHF20.
Finished cell lines:
H1792 expressing tagged p53
H1792 expressing tagged p53 and knock down of PHF20

Cell culture experiments

PHF20 knock down: is done with a PHF20 targeting shRNA . A virus containing the shRNA is generated as vector. The knock down is verified by western blot [7]



Metabolic labeling: The wild type cells as well as the knock down cells are fed with alkynyl-acetate analogs



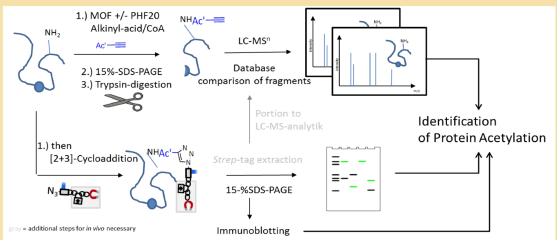
The optimal feeding time period and concentration has to be determined via fluorescent detection.

In vitro Experiments

To investigate whether p53 acetylation at lysine 120 by MOF is influenced by PHF20 in vitro the following reaction mixtures are prepared (Diagram step 1):
1) Control: MOF + H4K16 + Substrate [7]
2) MOF + p53 + Substrate
3) MOF + p53 + PHF20 + Substrate
4) MOF + p53 DBD + PHF20 + Substrate

Lysine 120 lies within p53s DNA binding domain, it is interesting to investigate if acetylation occurs just on a separate domain

Three approaches serve for detection of acetylation of p53:
1) LC-MS^n (the reaction mixture is separated via SDS, then trypsin digested before injecting to the system.)
2) Fluorescence: Here the click chemistry is used to add a fluorescent tag onto all acetylated sites. The mixture is separated over SDS PAGE and the gel can be analysed directly
3) Immunoblotting is also used where antibodies are used to mark acetylated p53



Conclusion

PHF20 might be involved in acetylation of p53 K120 through MOF and will be clarified in this project. The necessary chemical compounds and proteins will be synthesized through straightforward routs and protocols using the combined skill set of synthetic chemistry and molecular biology.
In vitro the performance of MOF will be monitored prior to experimenting with and without PHF20 in the acetylation mixture. Analysis will be carried out with three methods. Detection via fluorescence-marker in cooperated through click-chemistry, digestion and fragment analytic via LC-MS^n and finally through immunoblotting.
Experiments in cell culture use a knock-down PHF20 cell line as well as a wild type PH20 cell line. The click-chemistry approach is used to separate the labeled proteins from the matrix. The analytic can proceed as in the in vitro experiments.

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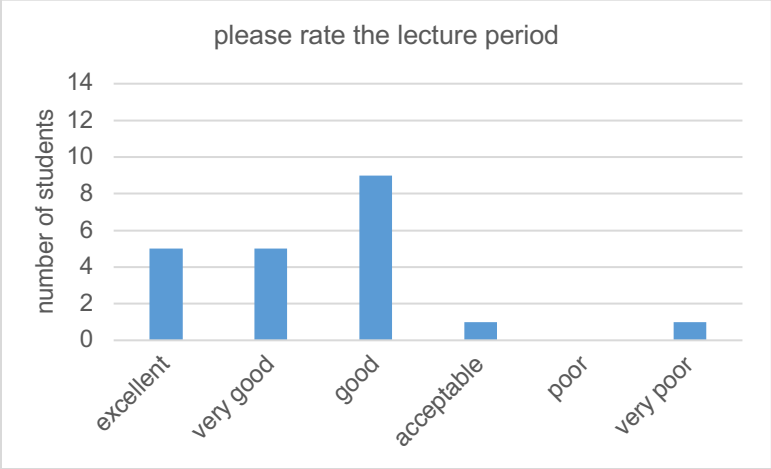
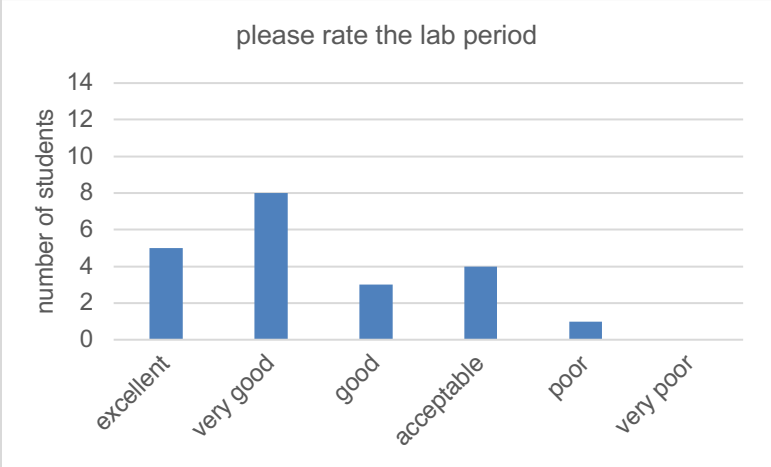
EVALUATION RESULTS

At the end of the course, the students were asked to evaluate the course. We used Likert scale and open-ended questions to collect the answers. The results and answers from the cohort in 2016/2017 and 2017/2018 were combined and are represented in the following. In the open-ended questions, the answers of the individual students were marked in italic from a to l.

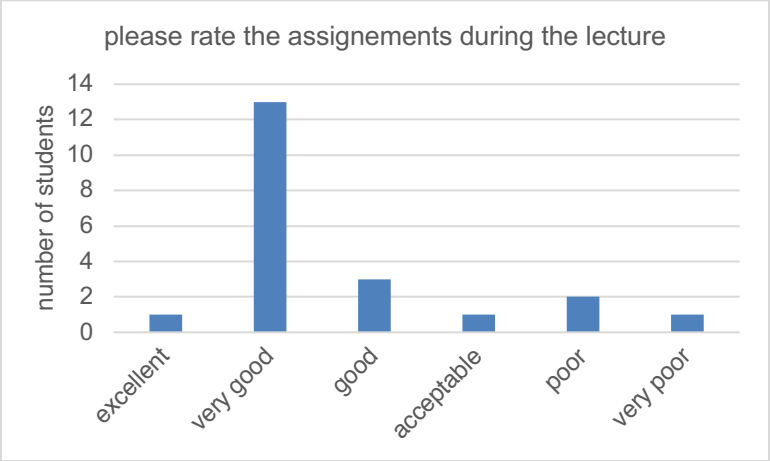
1) Please rate the lecture period



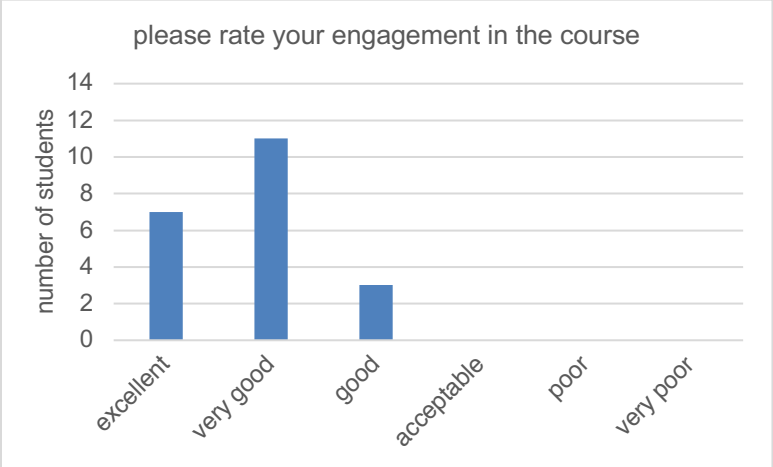
2) Please rate the lab period



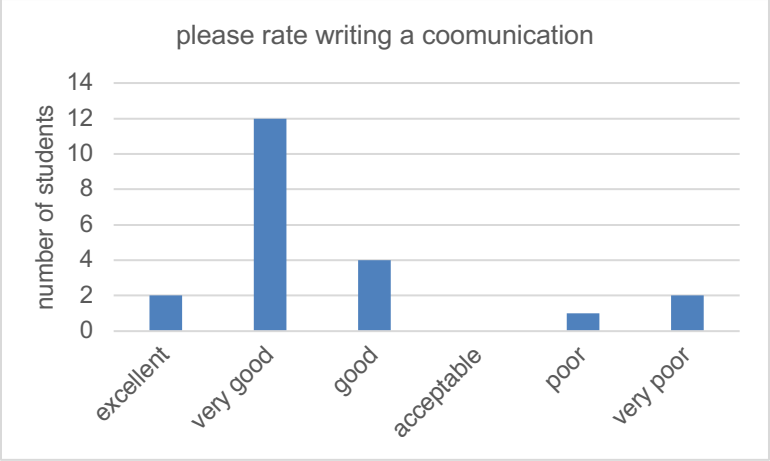
3) Please rate the assignments during the lecture



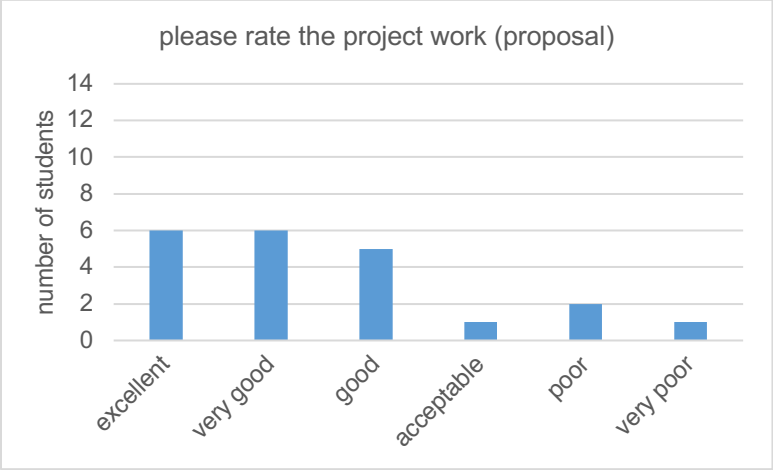
4) Please rate your engagement in the course



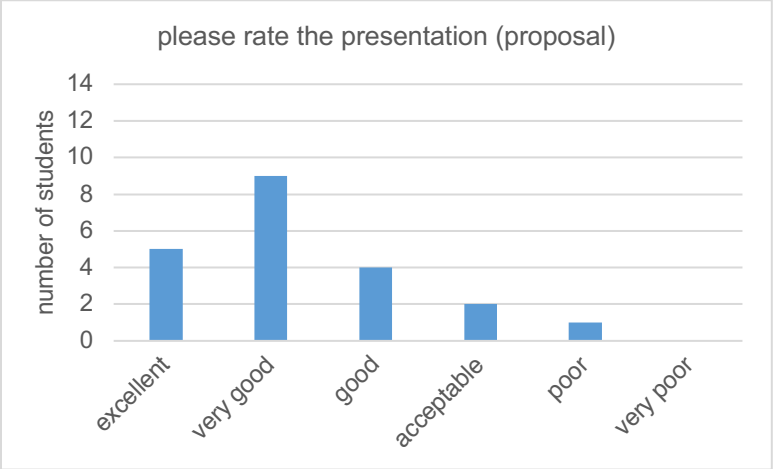
5) Please rate writing a communication



6) Please rate the project work (proposal)



7) Please rate the presentation (proposal)



8) What do you think was the most valuable part of the course for you personally?

a. *Learn how to write scientifically and communicate.*
b. *Doing the proposal.*
c. *Communication.*
d. *The lessons about the scientific writing.*
e. *Interacting with students of other disciplines and the writing of a paper and a communication.*
f. *To work interdisciplinary and to get to know a new research field.*
g. *The whole course felt more like a group project, which is very nice. We got to learn how to work as a group on different topics in the lab course and designing the fictitious project*

was a fun and also very valuable experience, because finding a research gap is a very important skill of scientists that isn't really trained during other courses.

h. *I really like to have the interdisciplinary part of the course. Working together with chemists and biologists was really interesting and I got some new insights in some biologic work and thinking.*

i. *This course really guide us to know how real research works (writing a paper, design experiment, developing ideas, create and present poster). Discussing ideas within an interdisciplinary group was also a great experience.*

j. *Refreshing some basic chemistry not used often in biological courses outside of mandatory chemistry lessons at the start of the bachelor. Additionally the use of language when writing scientific articles or protocols. It is expected of the students to write in English but the specific terminology is never focused on in any of the usual courses.*

9) Can you describe your experience interacting with the other students?

a. *Was a lot of teamwork, we wrote a lot, skyped, the others were fine. We helped us, was a good atmosphere.*

b. *Very good, one of the best thing of the course.*

c. *Very productive and friendly working environment.*

d. *Interaction was very valuable as well, because it felt like all students had different backgrounds and knowledge that could really come together in the proposal.*

e. *It was really nice to work in groups and get new contacts and discuss also with other students from another department and see their point of few to different themes.*

f. *It was a really great experience to discuss the ideas, the project with the teammate. It really push us to think how we can make the project better, we really complete each other and managed to create a project for the poster.*

g. *Everything went really well, everyone was helping each other to understand the lectures, the lab work etc.*

10) What was the biggest challenge for you in the course?

a. *Writing and formulating the thoughts about the research for everybody in the course understandable.*

b. *Making a somewhat complete story out of the course to write the communication about.*

c. *Time.*

11) What would you recommend, what we should definitely keep in the future?

a. *The lab work and Poster session.*

b. *Communication, Proposal, lab course and maybe theoretical lessons within the semester.*
c. *The combination of writing a communication and presenting the proposal as a poster.*
d. *Mixing chemistry and biology students, since we see the things from "the other side".*
e. *The way that you interact with the pupils.*
f. *The writing part was good. Keep that.*
g. *Changing the groups - although it is sometimes hard, but this is how it's going to be in the future, we cannot always choose who we are working with, so it really close to reality. Also the poster - much work, but definitely worth it because I learnt so much about the other discipline and chemical biology and had the chance to ask any question that came in my mind and discuss it in the group.*

12) To what extend do you think, did the course change your perspective on interdisciplinary research?

a. *It reinforced my opinion on the importance of interdisciplinary research.*
b. *It will definitely. I saw the power of interdisciplinarity. Also it was very inspiring during the work on the proposal.*
c. *It changed it, I am now not as "afraid" as before to read a chemical paper and I learned and read about chemical methods which I didn't have in my head as helpful for biology.*
d. *Beside the benefits of different point of view as well as different methodology for the same research question, communications between the fields seem to be a bigger topic than I thought.*
e. *I would like to work in an interdisciplinary research field in the future.*
f. *Interdisciplinary research is the future science.*
g. *The course got me really interested on the chemical biology and even more on the epigenetic topic and by designing our own project, we got to get an understanding of how you could apply knowledges from both disciplines.*
h. *I have learned new things and other ways of thinking.*
i. *Before I didn't pay much attention to the application of chemical methods for biology but the course introduced me to the ideas of using small molecules and chemical reactions in biological systems.*

13) To what extend do you think, did the course change your perspective on scientific writing?

a. *I really learned what "keywords" are useful in scientific writing. This helped a lot.*
b. *It has improved during the course a lot, because now I understand how is a paper structured and where to get the information. How to argue and present myself and my results.*

c. *It clarified the processes of writing and gave strategies not only for writing but also for reading.*

d. *A lot. It was always something I was scared of but now after finishing this course, I think that with practice it will get a lot better.*

e. *More self-confident.*

f. *No one has ever taught us the method of scientific writing. This is my first contact with and learning about science writing. Thank you very much.*

14) Do you think that this course is different from what you have done before? If yes, what are the main differences?

a. *The overall system is way better than normal lab courses or lectures, especially the poster session is a new part, because one has the possibility of thinking about new ideas and not repeating old scripts.*

b. *Yes. The interaction between students and the teachers were much more intensive. Somehow for me it was like giving a sense for what I am actually doing, it increased my motivation a lot. The combination of Theory, practical stuff and in the end the communication was in my point of view perfect.*

c. *A lot of teamwork.*

d. *Yes! More interactive working, learning of scientific writing, theoretical and practical work together with the same content.*

e. *Yes, the active involvement of the students was bigger. The interconnection between practical course and lab was new.*

f. *Yes it is. Different approaches with students from chemistry and biology working together and different theme (dealing with how to write scientific texts in a proper way).*

g. *Yes, it felt more like a real world project, more like we are actual scientists. I really enjoyed actually using my knowledge and not just following instructions!*

h. *It was different in that part that chemists and biologists work together in groups and see the different ways of thinking, which are varying between the two sciences.*

i. *Yes. Before we only knew how to write protocol, which actually in real research we are not going to write protocol, but mostly paper, poster, and presentation.*

j. *Yes, a lot more team work, a lot more support of the professors and supervisors.*

k. *Yes, real improvement in skills and learning. In the process of completing the poster, I really understood how to do scientific research by discussing and reading the literature.*

l. *Yes, usually in courses the students either work alone on a topic or in set groups that won't change during the course. The constant changing of groups allowed to work with different people throughout the course and related subjects.*

15) Does this course changed your perspective of biology and chemistry as separated disciplines? Why?

a. *During the course I really experienced how colleagues from other departments contributed to the way of solving a problem. It was nice and inspiring to see their ways of approaching a problem with their methods.*
b. *Yes, it all depends more than I thought.*
c. *Yes, a lot.*
d. *I think they are very much connected and you can't look at them as separate disciplines.*
e. *Yes, because you learns how to combine both.*
f. *I don't think so. On the contrary, for the first time, I realized how interdisciplinary research links two different disciplines. Although the course was over, I continued to read the literature in the related field and the book the professor had recommended because it benefited a lot.*
g. *No, before I also thought it is not a great gap between biology and chemistry.*

16) Do you think that both divisions (chemistry and biology) can work in a synergistic way? Why?

a. *Sure they can... biologist work with "living" systems they observe the whole organism, they have a broader view on things... but when it comes to molecular levels chemists can explain things a lot more in detail.*
b. *Yes because they can complement each other really well.*
c. *Of course, better overview of the whole subject, resulting in a better understanding.*
d. *Yes, because there is a need of new perspectives to solve.*
e. *I think the synergistic way is the only way for future.*
f. *It feels like the biggest research topics are in medical science/biology. By applying chemical methods like synthesis or analysis methods, you can really help to solve problems that are not as easy from the biological standpoint.*
g. *Yes, because the two disciplines are really close and can learn a lot from each other. Problems can be solved in the one discipline with the help of the other.*
h. *Yes, because the challenges are seen from different ways.*
i. *Of course they can, there is basically no real separation and if both parties are enthusiastic and able, the cooperation will bear rich scientific yield.*
j. *Yes, it is proven already by our research proposal (poster) that we can solve problem with chemical biology approach.*
k. *Yes, I think not only chemistry and biology can work together, but even more cross-cooperation with pharmacy or physics. For example, the intracellular binding sites can be better detected by different electron microscopy methods. Many problems in biochemical synergy research can solve pharmaceutical applications.*

l. *Yes. During the development of the research proposal has shown that we often approached a task from different perspectives. The biologists were often focused on the 'bigger picture', which part of a biological system to target or which organism for example. The chemistry students however were very helpful in finding the specific molecule and the chemical processes required to target them.*

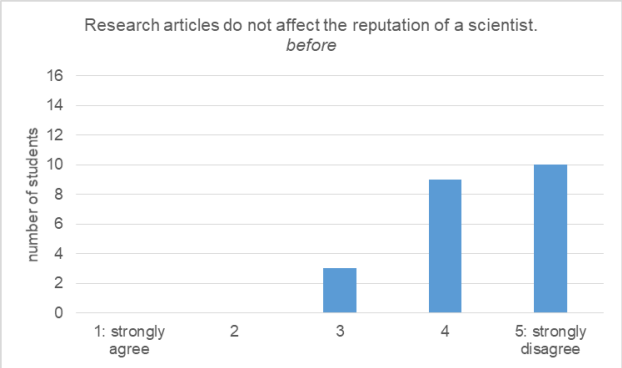
17) Do you have the feeling that interactions and problems solving between chemists and biologists went in both directions or just in one?
    a. *In both directions.*
    b. *In both.*

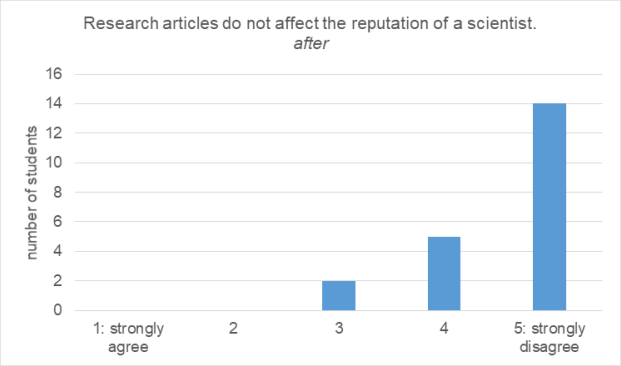
18) Do you think that writing the communication give you a better overview than the traditional separate protocols? Why?
    a. *Yes, a communications gives one a "big picture" of a research project.*
    b. *Yes! By writing a paper I learned a lot about the way of doing research, informed and get an overview of the recent research topic.*
    c. *Yes because you were confronted to really make something out of the data we got and not just write it down and if something didn't work just write that also.*
    d. *Yes because it's not just writing down the results, it is a kind of presenting the results.*
    e. *Yes it does since one is forced to think a bit more about what was done in the course.*
    f. *Yes, communication gives you an overview about the problem and how you solve it.*
    g. *Yes. In separate protocols you only concentrate on the results you get and what they mean. In a communication you also have to combine everything in a bigger picture.*

NATURE OF SCIENTIFIC PRACTICE SURVEY RESULTS

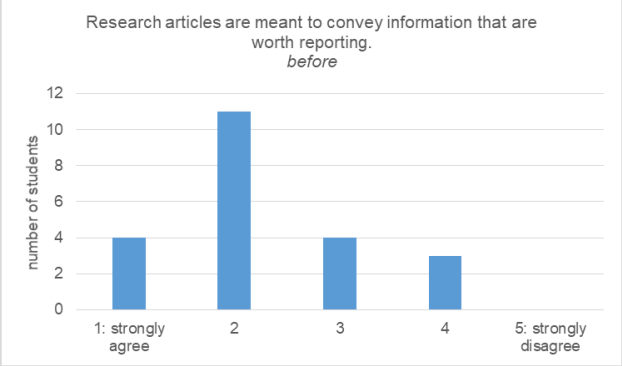
The students performed a nature of scientific practise survey in the beginning and at the end of the course. The results from before and after the course are represented in the following:

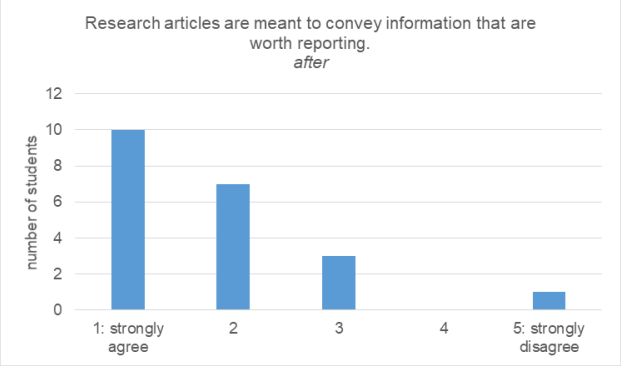
1) Research articles do not affect the reputation of a scientist.



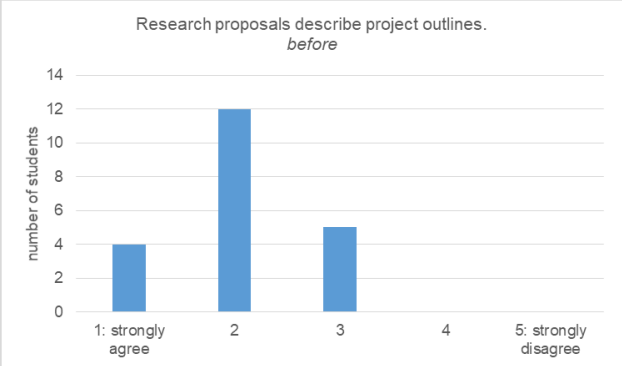


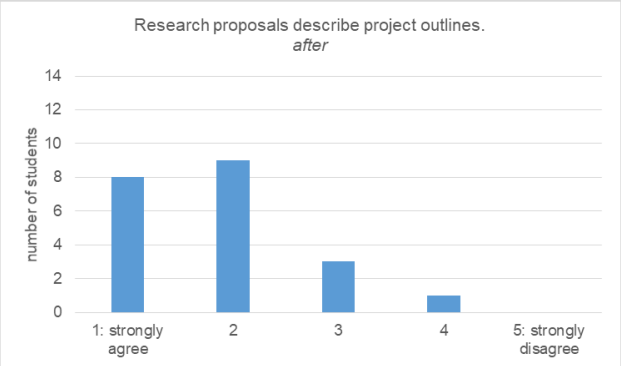
2) Research articles are meant to convey information that are worth reporting.



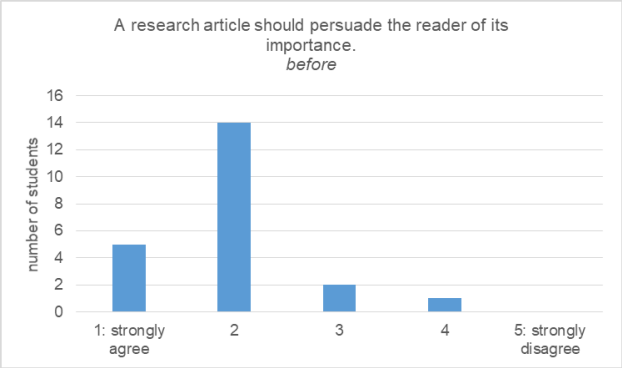


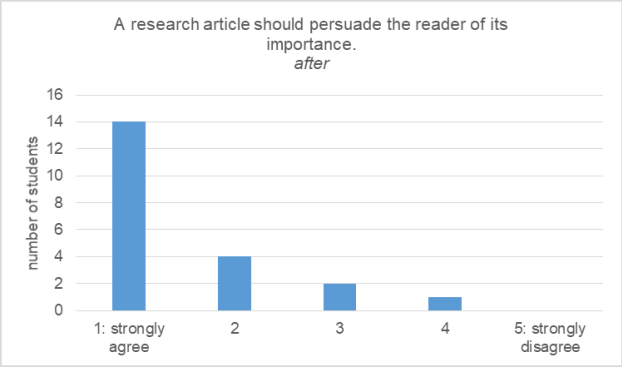
3) Research proposals describe project outlines.



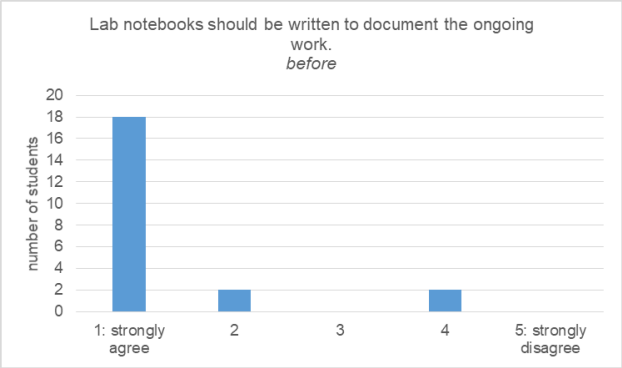


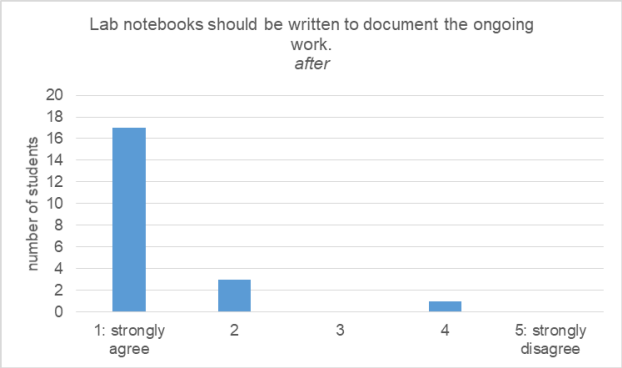
4) A research article should persuade the reader of its importance.



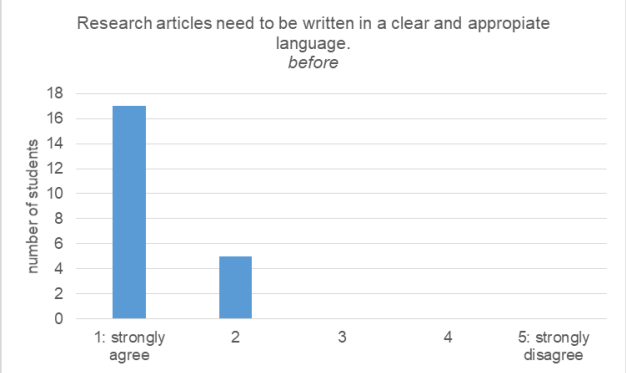


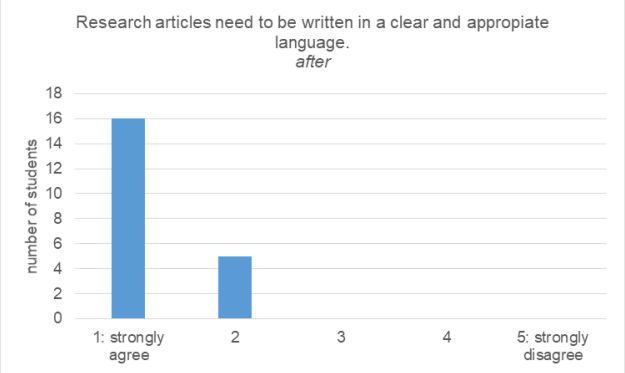
5) Lab notebooks should be written to document the ongoing work.





6) Research articles need to be written in a clear and appropriate language.

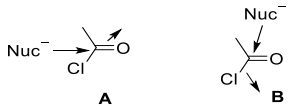


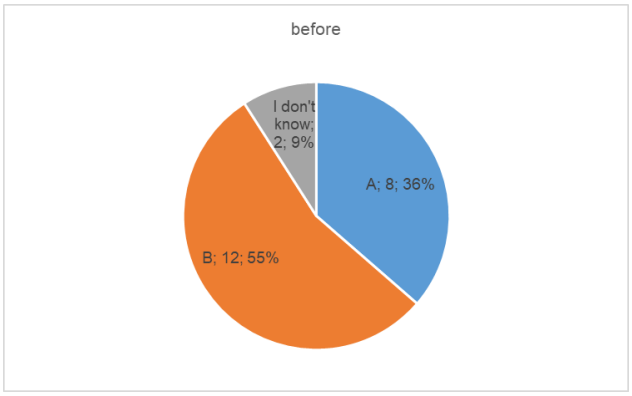


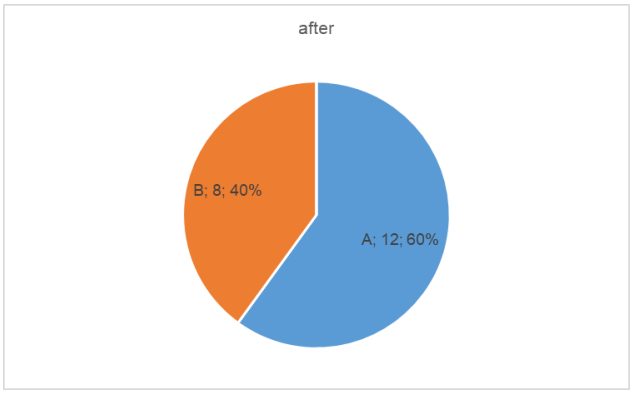
ORGANIC CHEMISTRY SURVEY RESULTS

The students performed an organic chemistry survey in the beginning and at the end of the course. The results from before and after the course are represented in the following:

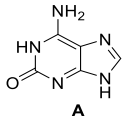
1) Indicate the direction of the reaction step.

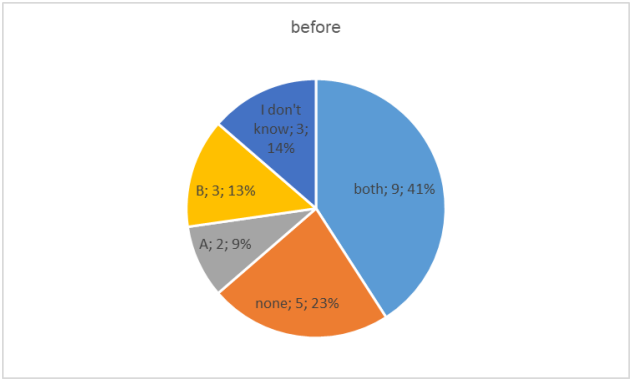


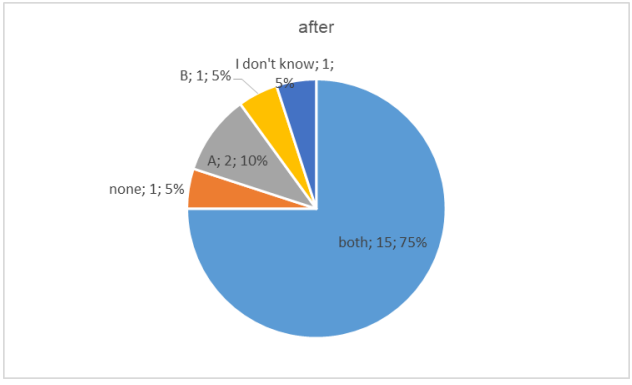




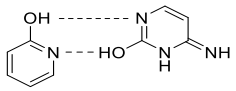
2) Which molecule is aromatic?



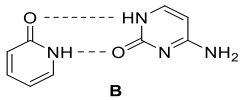




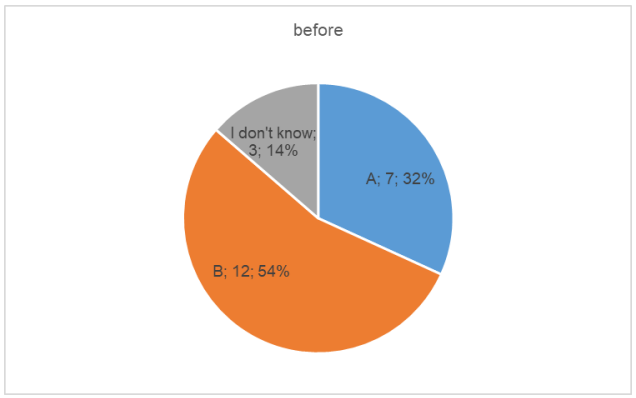
3) Which interaction is more favoured?

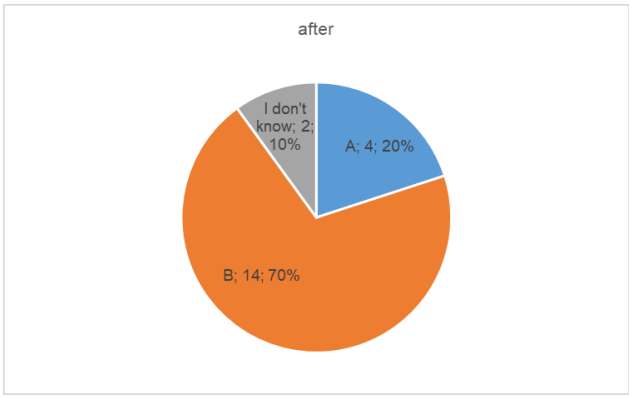


A



B





4) Which property are you expecting from the amino acid lysine?

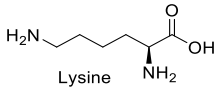
a. Acidic

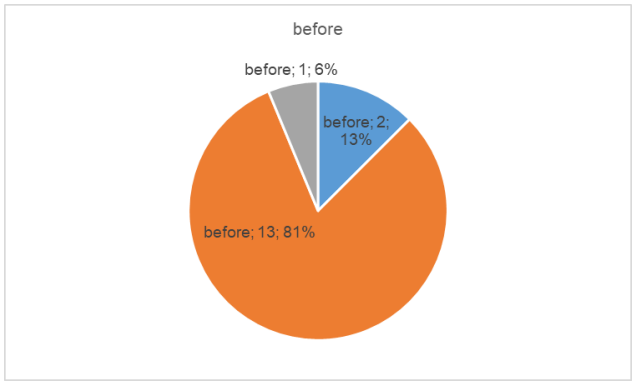
b. Basic

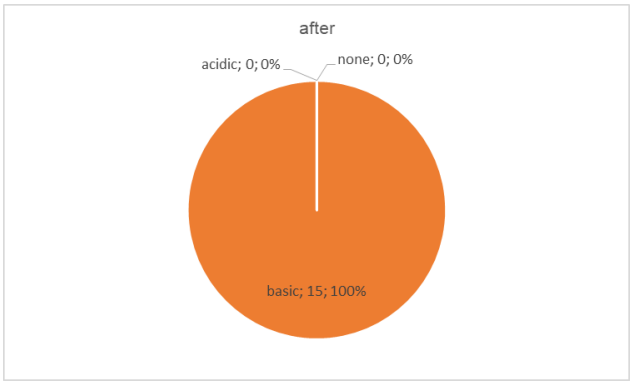
c. Nonpolar (hydrophobic)

d. Polar (uncharged)+

e. Other







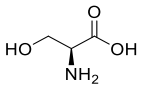
5) Which amino acid is shown in the picture?

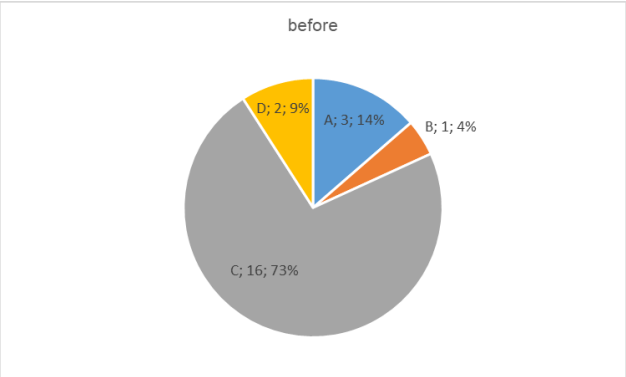
a. Cystein

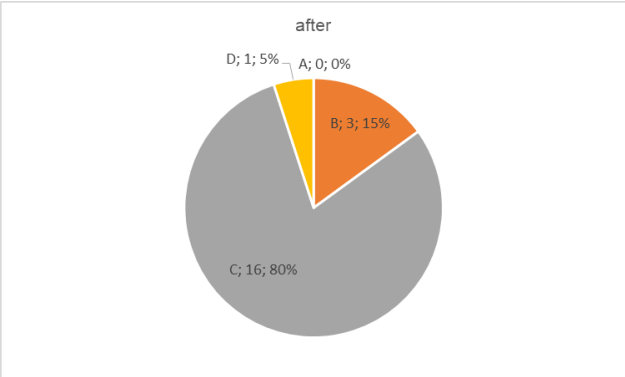
b. Methionine

c. Serine

d. Other







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