Reading School of Pharmacy

University of Reading

POLYMER-DRUG CONJUGATES AS NON-PRODRUG SYSTEMS, A STRATEGY OFFERING PERIPHERAL LOCALISATION OF DRUGS AND RETAINED BIOLOGICAL ACTIVITY

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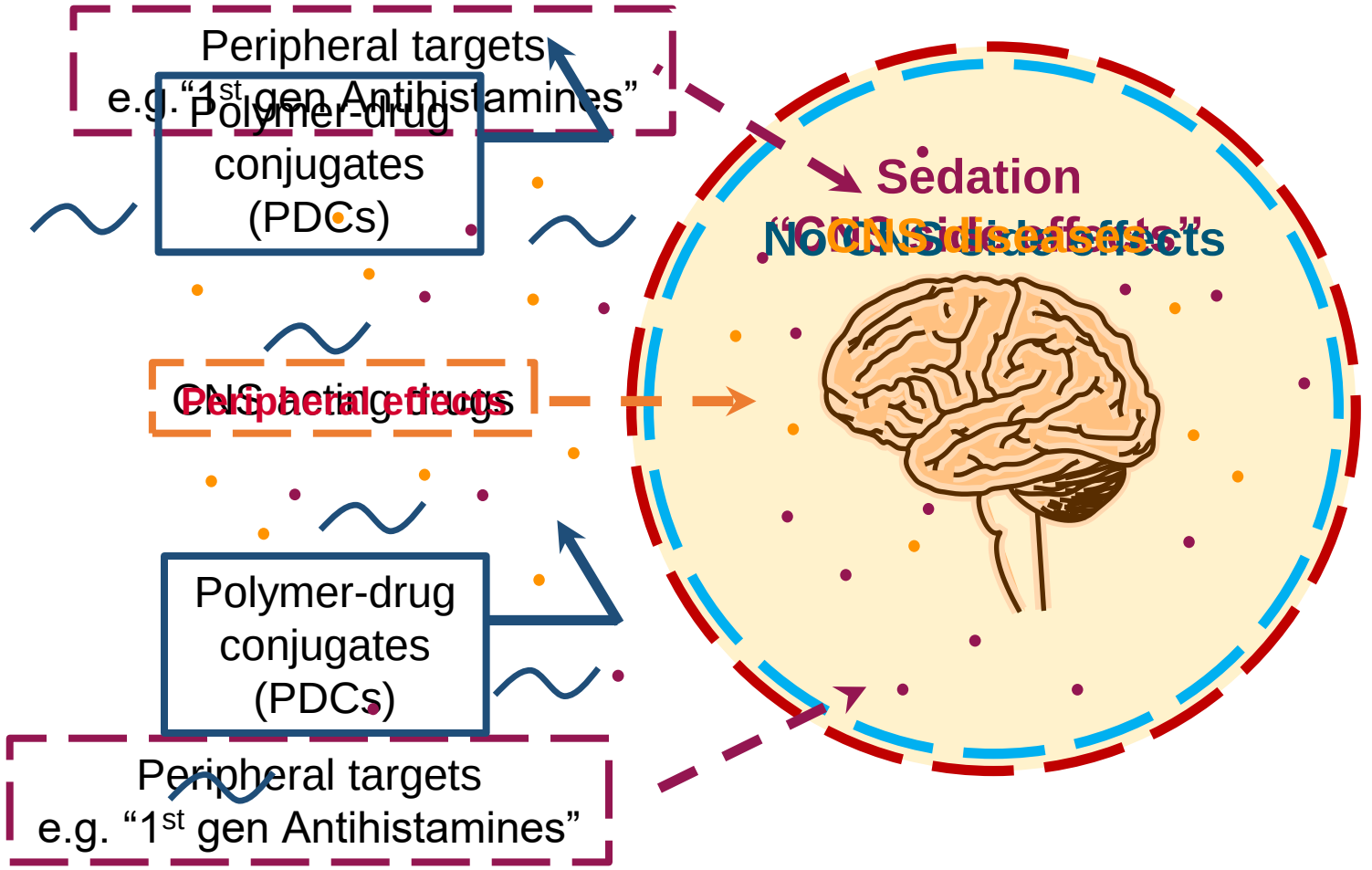
Background

The blood-brain barrier (BBB) & drug delivery

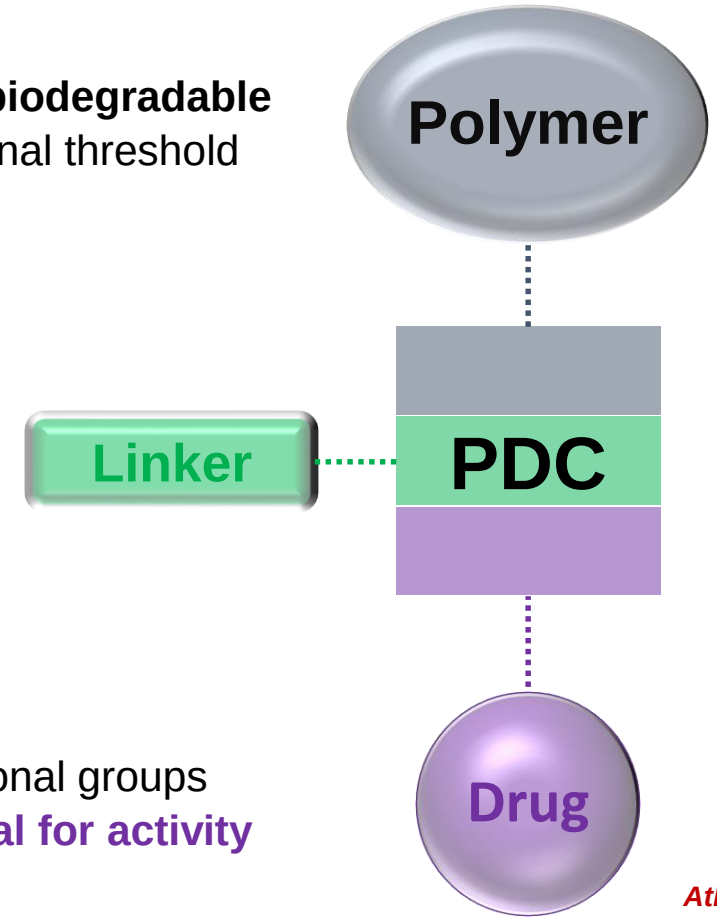


Bicker et al, Eur. J. Pharm. Biopharm . 87 (2014) 409-432

Background

The concept of PDCs

Non-biodegradable
<Renal threshold



Biologically **Stable**

With functional groups
NOT essential for activity

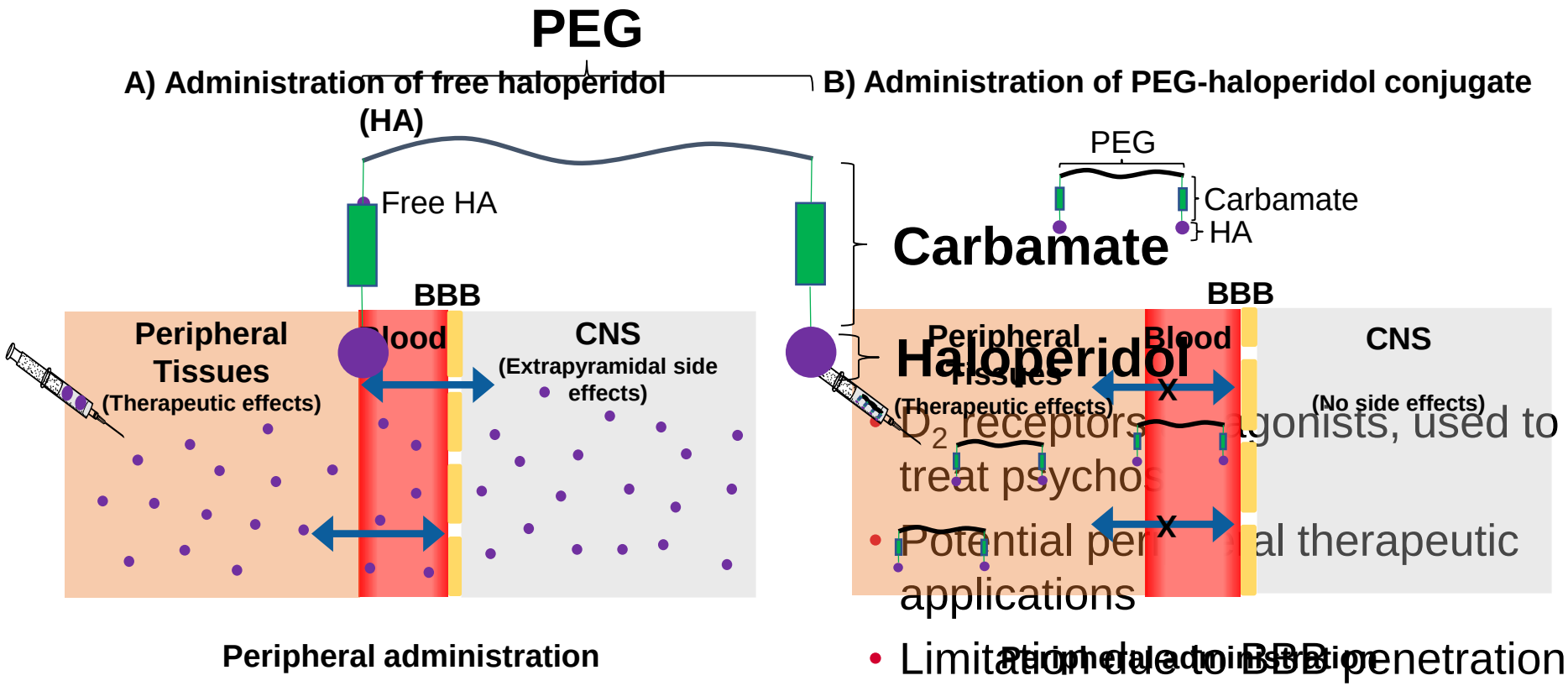
Peripheral localisation of PDC with retained activity

Atkinson et al, J. Pers. Med. 8 (2018) 1-24

Natfji et al, Curr. Opin. Colloid Interface Sci. 31 (2017) 51-66

AIM

PEG Haloperidol Conjugate BBB



- D_2 receptors antagonists, used to treat psychos
- Potential per al therapeutic applications
- Limitation due to BBB penetration

Heath et al, Polym. Chem. 7 (2016) 7204–7210

Objectives

1) Synthesis and characterisation of PEG-HA conjugate

RP-HPLC and **NMR**

2) Assessment of the retained biological activity of PEG-HA

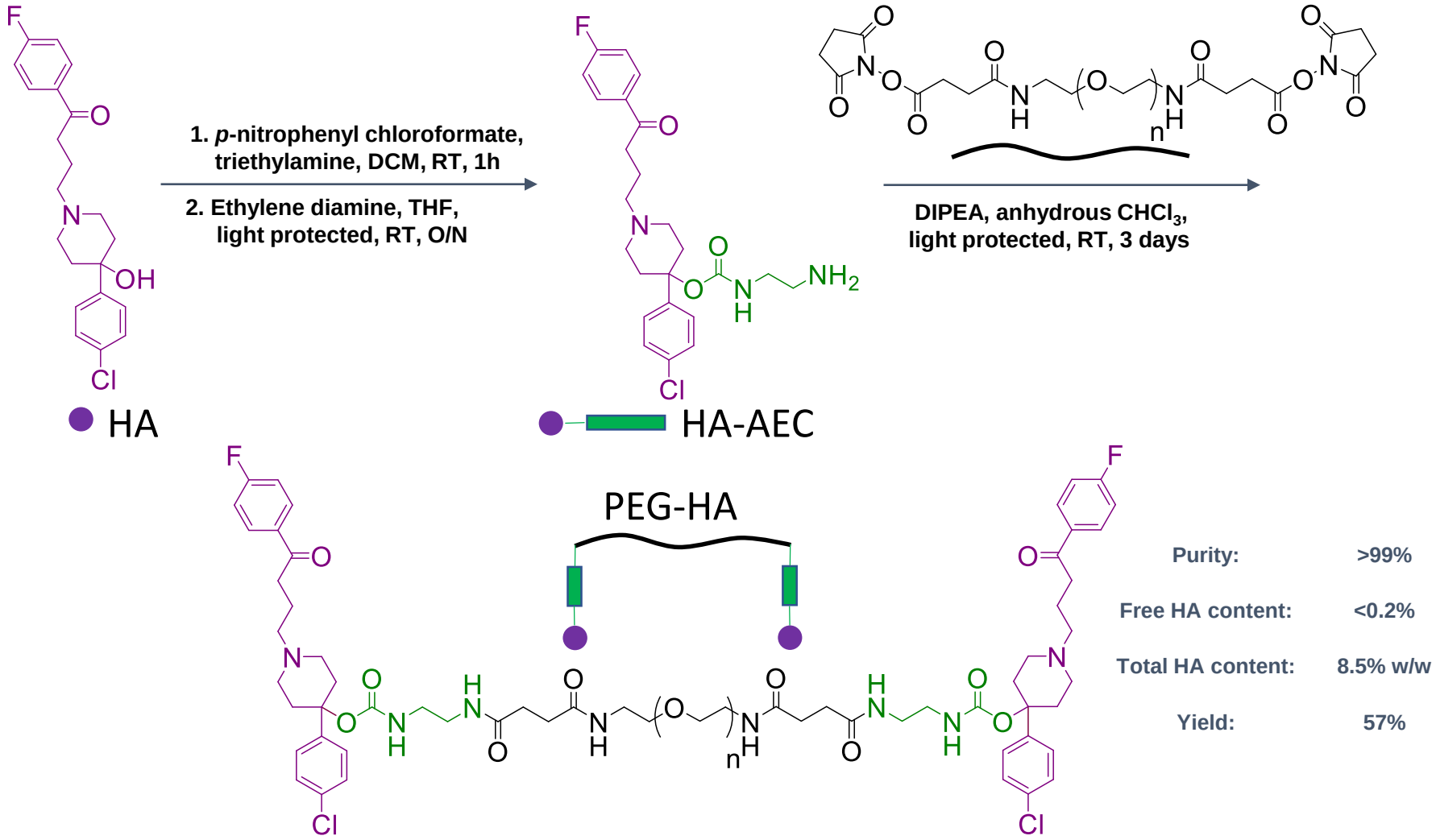
In vitro *via* **[^{35}S]GTPγS-binding assay**

3) Evaluation of impaired penetration through the BBB

In silico prediction of **log BB** value of PEG-HA

In vivo by recording **catalepsy** in rats

Results: Synthesis and characterisation

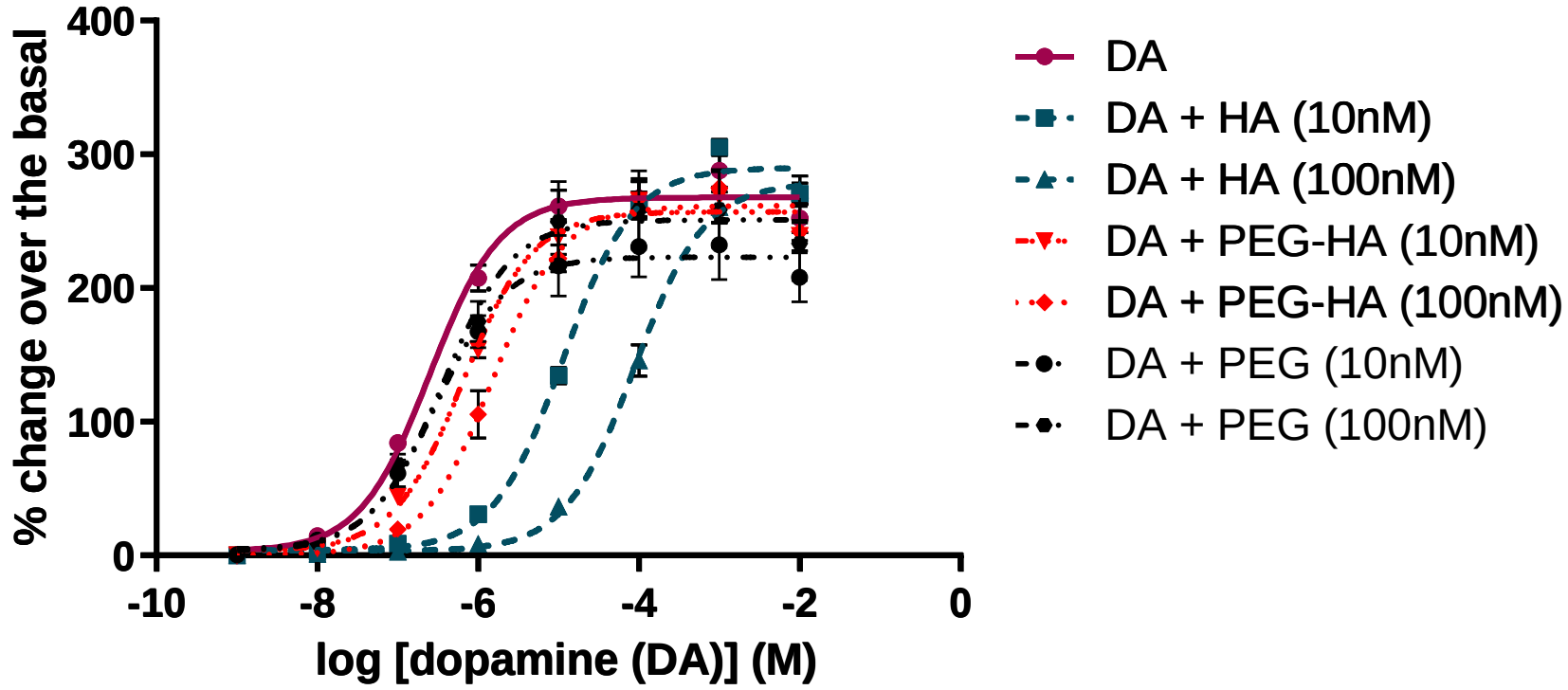


Purity:	>99%
Free HA content:	<0.2%
Total HA content:	8.5% w/w
Yield:	57%

Natfji et al, submitted

2) Retained activity on D_2 receptors

In vitro [^{35}S]GTPγS-binding study



Using D_2 receptors from CHO cells, n=3, mean ± s.e.m

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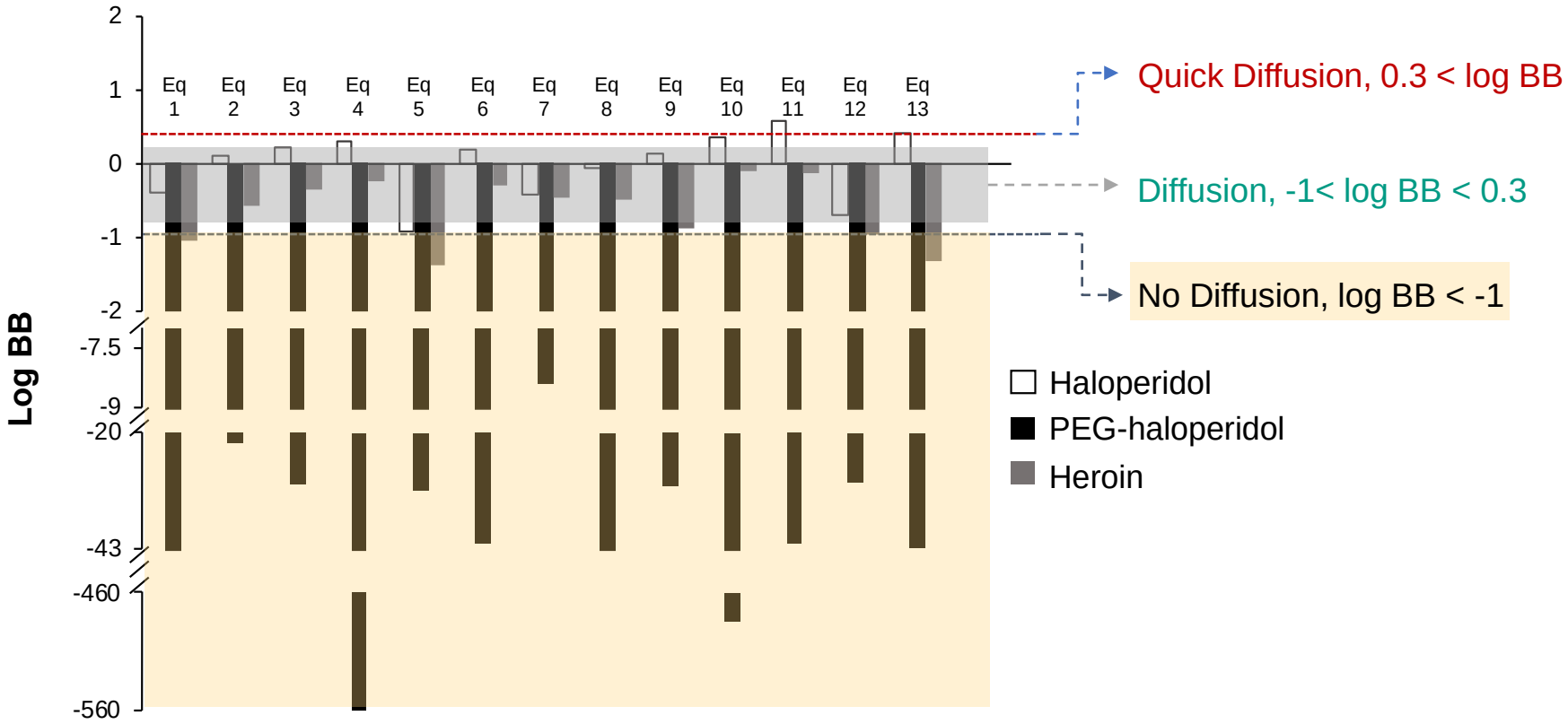
3) Peripheral localisation

In silico prediction of log BB values



Natfji et al, submitted

3) Peripheral localisation

In vivo BBB permeation study (the catalepsy test)

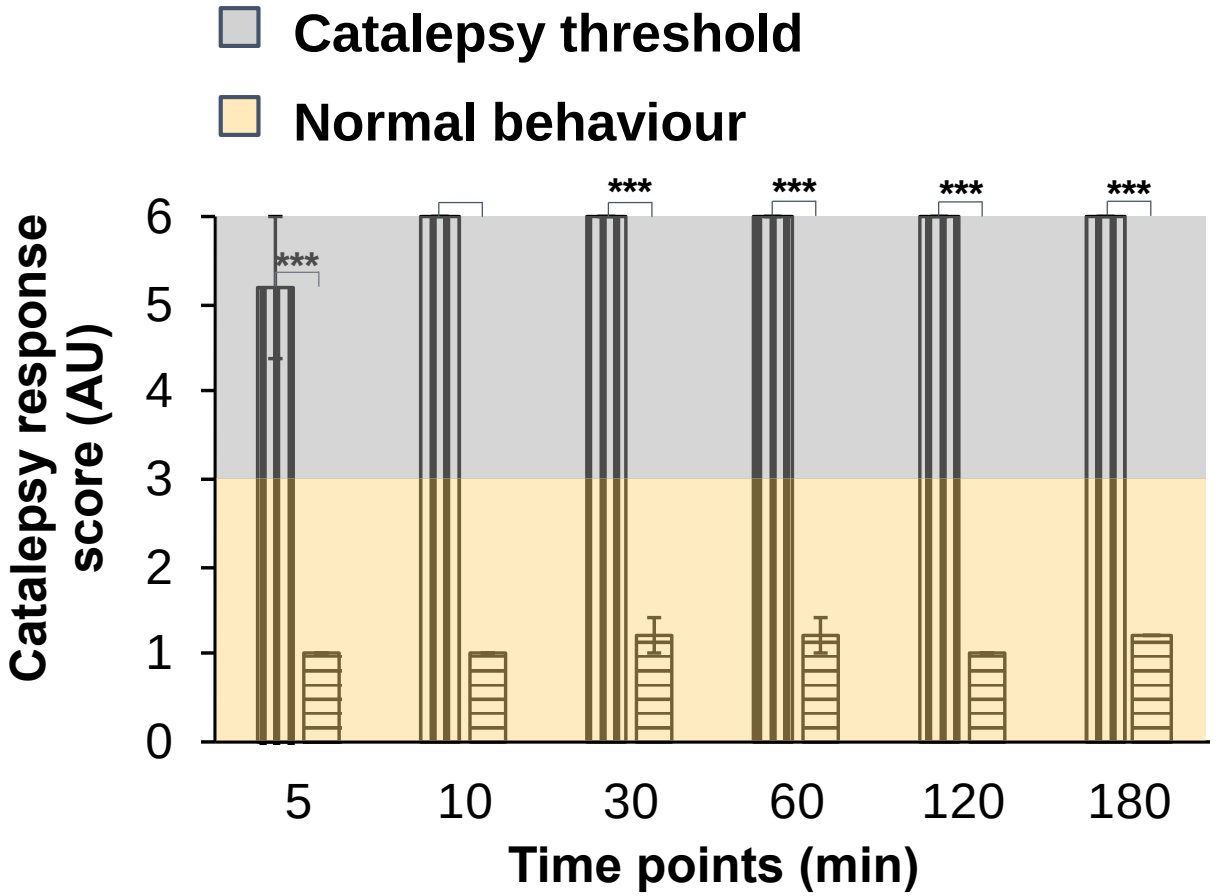
Catalepsy in rats is the inability of animals to correct immobilised postures.

Recording **catalepsy** in animals is used to predict the **extrapyramidal side effects** of HA due to the permeation through the **BBB**.

I.v. free HA (1mg/kg)	*I.v.* PEG-HA (1mg/kg, HA equiv)
A cataleptic rat	**Normal behaviour**
	

3) Peripheral localisation

In vivo BBB permeation study



Catalepsy scoring system	
Time on Bar	Score
0-10 s	1
11-20 s	2
21-30 s	3
31-40 s	4
41-50 s	5
51-60 s	6

Catalepsy recording test, n=5, mean ± s.e.m

Natfji et al, submitted

Conclusions

- PEG-HA conjugate was successfully synthesised and characterised.
- PEG-HA was significantly able to inhibit dopamine-induced activation *via* D_2 receptors.
- *In silico* study indicated PEG-HA is very unlikely to cross the BBB.
- PEG-HA did not induce catalepsy in rats after *i.v* treatment.
- PEG-drug conjugate, as a **non-prodrug**, offers potential for therapeutic applications for drugs where peripheral actions are desired, without inducing central effects.

Acknowledgment

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  Dr Felicity Heath, Amy Newman, Chiara Clementi, Prof Gianfranco Pasut

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  Prof Gary J. Stephens, Hong Lin

- ***In vivo* study:**

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Thank you

Any Questions?

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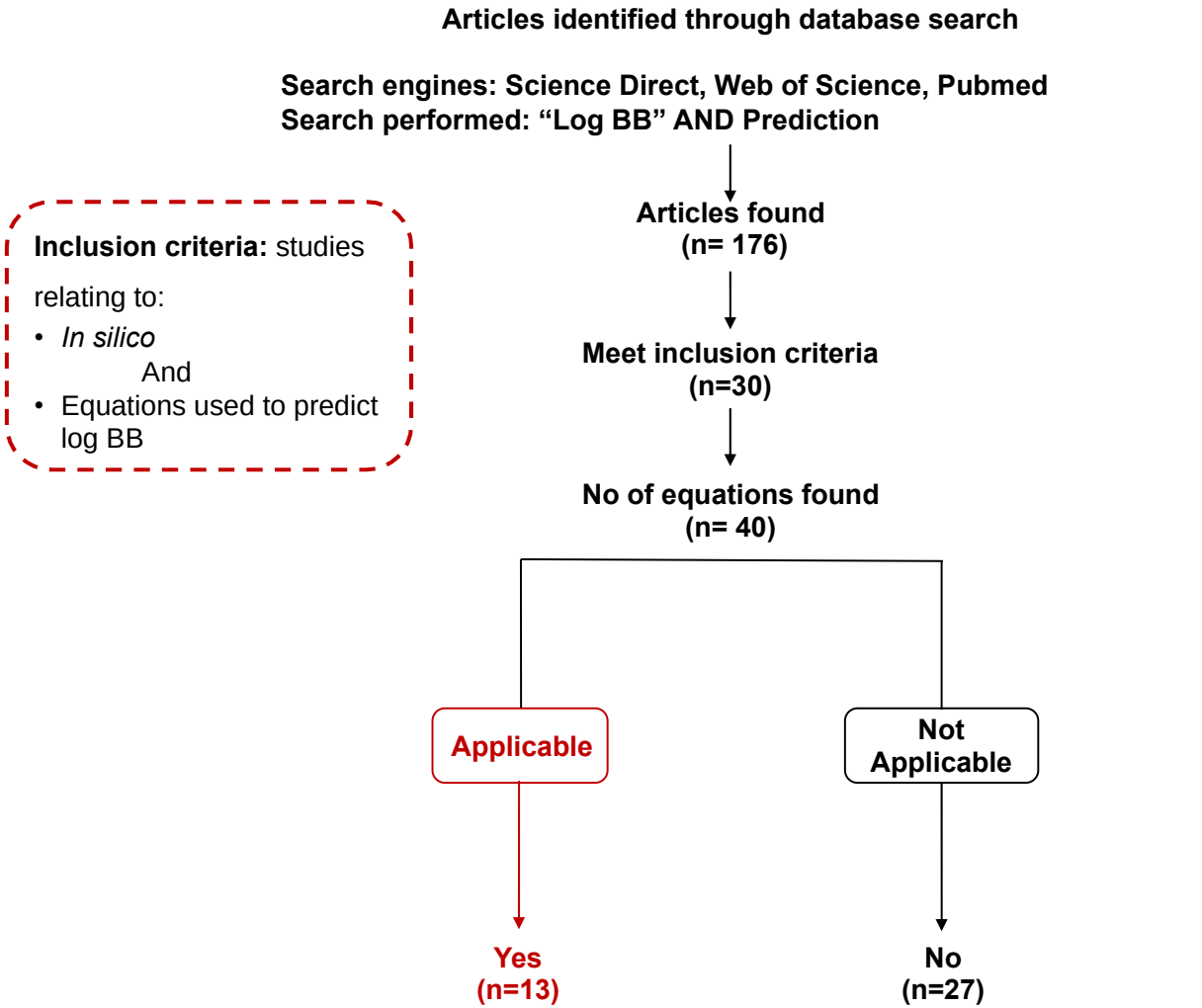
In silico prediction of log BB value



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Retained biological efficacy of PEG-HA conjugate

Drugsc	pEC_{50}
Dopamine	6.59 ± 0.07
Dopamine + HA (10 nM)	4.94 ± 0.04 ***
Dopamine + HA (100 nM)	4.05 ± 0.09 ***
Dopamine + PEG-HA (10 nM)	6.19 ± 0.02 *
Dopamine + PEG-HA (100 nM)	5.81 ± 0.11 ***
Dopamine + PEG (10 nM)	6.52 ± 0.06 ns
Dopamine + PEG (100 nM)	6.39 ± 0.09 ns

Natfji et al, submitted,

In silico prediction study of log BB

No	Equation	Log BB Heroin	Log BB HA	Log BB HA-PEG-HA
1	Log BB= 0.4275 - 0.3873($n_{acc,solv}$) + 0.1092(log P) - 0.0017(A_{pol})	-1.06	-0.4	-58.87
2	Log BB=-0.0148PSA + 0.152ClogP + 0.139	-0.59	0.095	-22.02
3	Log BB=-0.0145PSA + 0.172 Mlog P + 0.131	-0.38	-30.32	0.22
4	Log BB=-13.31V^2 + 9.601V - 2.231PSA - 0.529	-0.26	0.29	-593.9
5	Log BB= -0.00075 Vm-0.019 PSA	-1.38	-0.92	-31.38
6	Log BB = -0.021 SP - 0.003Vm + 1.643	-0.32	0.18	-42.01
7	Log BB= -0.00116MW + 0.272 log P - 0.088	-0.47	-0.44	-8.42
8	log BB= -0.01Vm + 0.35 log P + 0.99 I3 + 1.25	-0.49	-0.08	-67.34
9	Log BB= 0.2[(logP-(N+O)]	-.89	0.13	-30.81
10	Log BB=-9.880x$10^{-6}$$MW^2$+7.339x$10^{-3}$MW-0.2268$n_{pol}$-0.1143	-0.11	0.34	-485.28
11	Log BB= 1.045 + 0.138 logD7.4 - 0.292 HBA	-0.14	0.58	-42
12	Log BB= - 0.2339 HBAC + 0.00147 MVOL x 31.6099x HBACxHBDN$^{1/2}$/SASA - 0.04579	-0.98	-0.7	-29.66
13	Log BB= 0.5159X log$P_{o/w}$ – 0.0277xTPSA-0.3462	-1.35	0.42	-42.65

Abbreviation: logP: logPo/w: octanol/ water partition coefficient; ClogP: calculated logP; V: molar volume, nm^3; PSA: polar surface area, nm^2; PSA: polar surface area; N+O: total number of nitrogen and oxygen atoms; ; MW: Molecular weight; n_{pol}: number of polar atoms (nitrogen, oxygen, and attached hydrogens); HBAC: number of solute hydrogen bond acceptor; HBDN: number of solute hydrogen bond donor; MVOL: molecular volume; SASA: solvent accessible surface area; MW: molecular weight; TPSA: topological polar surface area

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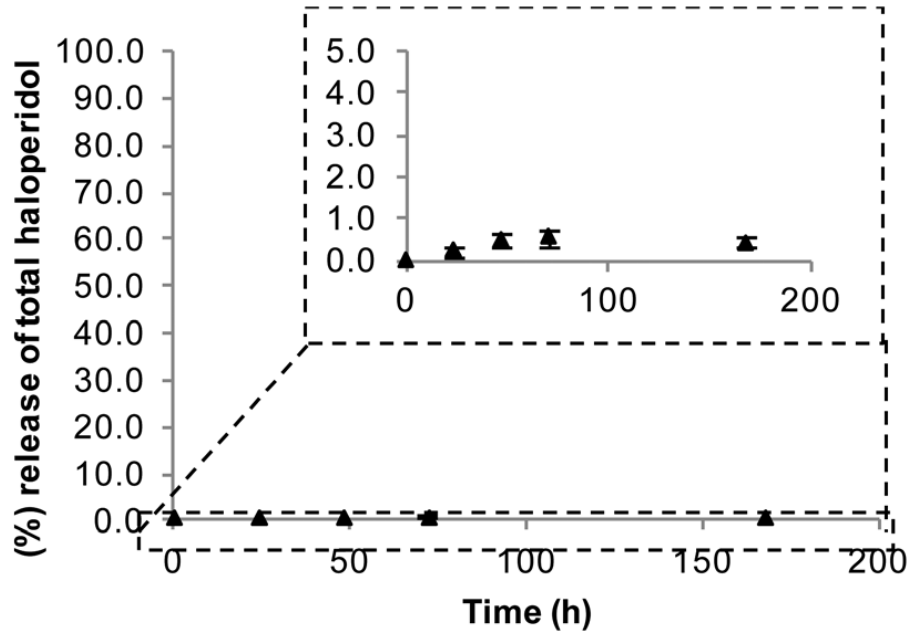
In vitro Stability study

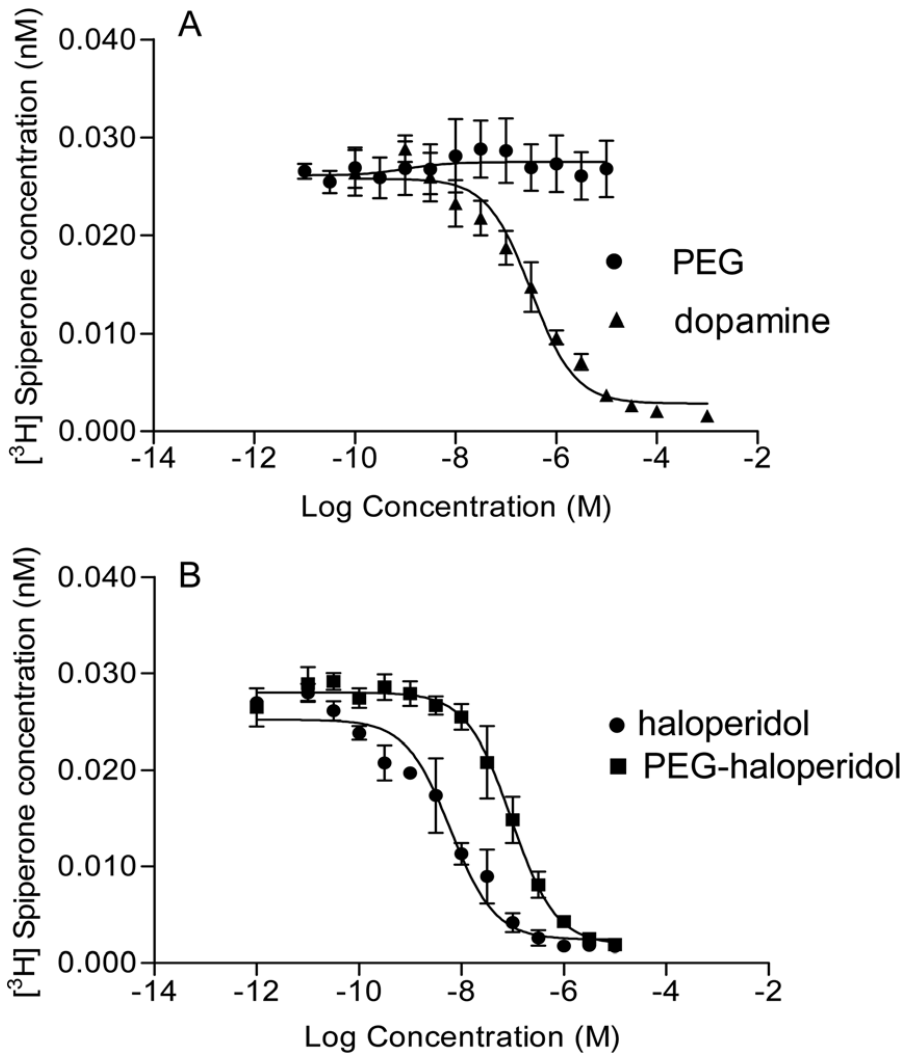
Stability of PEG–haloperidol conjugate on incubation with rat plasma. Data expressed as mean ± SEM (n = 3). Error bars hidden by the symbols when not visible.



Heath et al, Polym. Chem. 7 (2016) 7204–7210

Binding study

Binding curves for compounds competing with [3H] spiperone in D2 receptors from CHO cells: (a) PEG, negative control and dopamine, positive control, (b) PEG–haloperidol and free haloperidol. Data expressed as the mean ± SEM, (n = 3)



Heath et al, Polym. Chem. 7 (2016) 7204–7210