SMART MICELLAR SYSTEMS FOR BRAIN DRUG DELIVERY

Y. Islam[1], A. Leach[1], C. Coxon[1], J. Downing[1], M. Platt[2], S. Pluchino[3], T. Ehtezazi[1]

[1]School of Pharmacy and Biomolecular Sciences, James Parsons Building, Byrom Street, Liverpool, L3 3AF
[2] Department of Chemistry, Loughborough University, Loughborough LE11 3TU, United Kingdom
[3] Department of Clinical Neurosciences, Centre for Brain Repair, University of Cambridge, Robinson Way CB2 0PY, UK.

1. INTRODUCTION:

- Neurodegenerative disorders (like dementia, MS, PD, AD, stroke and traumatic brain injury) cost directly to healthcare system over £134 billion per annum in the UK and USA. These disorders affect the quality of life of the patient.
- Brain matrix metalloproteinase-9 (MMP-9) levels increase in neurodegenerative disorders. An enzyme that plays an important role in many pathological processes of brain injuries and inflammation.

The Challenge

- Conventional drug delivery systems have number of limitations such as route of administration (intracranial injection), bioavailability (permeability across the BBB), and the safety of the carrier material.

Our approach

- We aim to develop a ***safe and smart material*** that forms a micellar drug delivery system with the ability to target the brain specifically and release a payload at the suitable region. Our approach is to apply state-of-the-art integrated experimental and computational methods to identify the smart enzyme responsive materials (Figure 1).
- The key advantages of our smart biomaterial are; permeability across the BBB and being responsive to MMP-9.

2. METHODS:

- Experimental data was analysed by applying statistical models; Lamort, Kridel , Kcat/KM and Ratnikov datasets in relation to MMP-9 cleavable peptides.
- Molecular docking was applied to identify MMP-9 cleavable peptides with desired sensitivity.
- Synthesis of suitable peptides using a CEM Liberty Blue synthesizer, and characterization via Liquid chromatography–mass spectrometry (LC-MS).
- Conjugation of Cholesteryl chloroformate with peptide, using N,N-Diisopropylethylamine (DIPEA) and Dimethylformamide (DMF).
- Characterizing the self-assembled nano-micelles using transmission electron microscopy (TEM).

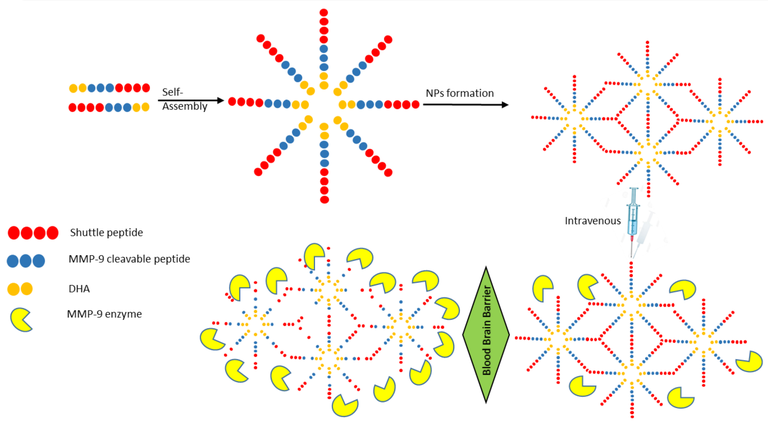


Figure 1. Schematic presentation of the core of the smart biomaterial and mechanism of action of Smart-MMP-9 Responsive drug delivery system..

Table 1. A list of known MMP-9 substrates, processed using statistical techniques to assign cleavability scores.

Sequence	Probability	Lamort	Ratnikov	Kridel	Kcat/KM	Y_Score
KAG-LLC	4.3636		0.47	-0.11	251	0.18
KG-LC	8.22765		0.47		358	0.47
SGRR-LLSRTA	0.51	3.61	0.50	0.12	1,200	1.41
RPLA-LWRSQ	4.12	4.03	0.69	0.49	2,000	1.74
RGLA-LWRSQ	0.36	3.37	0.56	0.47	2,000	1.47
GPLE-LEEAQ	29.73	4.06	0.55	0.45	2,000	1.69
EAAPSS-VIAATE	24.05	4.13	0.53	0.46	2,000	1.71
SGLRPAK-STA	23.23	3.74	0.64	0.00	5,800	1.46
PLG-LEEA	46.53	3.87	0.66	0.46	8,000	1.66
GPQG-IAGQ	1449.48	4.00	0.62	0.27	8,400	1.63
SGQPHY-LTTA	27.00	3.84	0.65	0.37	8,800	1.62
IPES-LRAG	38.64	4.07	0.66	0.36	12,600	1.70
SGFGSRY-LTA	52.20	3.76	0.63	0.11	13,200	1.50
GPLG-LERAQ	160.15	4.11	0.62	0.51	14,000	1.75
GAMF-LEAIP	6.26	4.06	0.60	0.26	15,000	1.64
RPLA-LEESQ	4.08	3.99	0.60	0.44	21,000	1.68
KPAG-LLGC	191.40	4.08	0.62	0.45	24,000	1.72
SGPLF-YSVTA	11.48	4.10	0.67	0.37	25,600	1.71
PLA-LWRSQ	5.64	4.03	0.68	0.49	51,000	1.73
SGPRA-VSTTA	74.98	4.17	0.65	0.35	61,000	1.72
RPLG-LEEA	34.02	3.86	0.66	0.46	62,000	1.66
RPLE-LWRSQ	1.59	4.02	0.63	0.45	67,000	1.70
SGKIPRT-LTA	52.94	4.16	0.68	0.20	67,500	1.68
RPLG-LGAAQ	237.75	4.23	0.74	0.38	133,000	1.78
RPLA-LWEEQ	9.19	3.96	0.71	0.39	143,000	1.69
GPLG-LREAQ	566.23	4.15	0.71	0.50	160,000	1.79
SGKIPRR-LTA	29.81	4.09	0.72	0.18	160,000	1.66
SGKGPRQ-ITA	170.54	4.15	0.65	0.23	188,000	1.68
RPLA-LWESQ	5.67	3.99	0.69	0.49	198,000	1.72
RPLG-LGGAQ	638.64	4.24	0.74	0.38	270,000	1.79
RPLA-LRRSQ	7.63	4.12	0.69	0.44	291,000	1.75
RPLG-LWGAQ	482.56	4.19	0.74	0.51	750,000	1.81

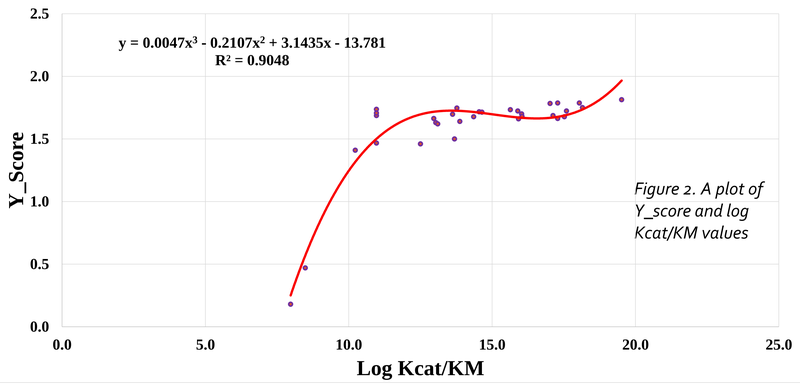


Figure 2. A plot of Y_score and log Kcat/KM values

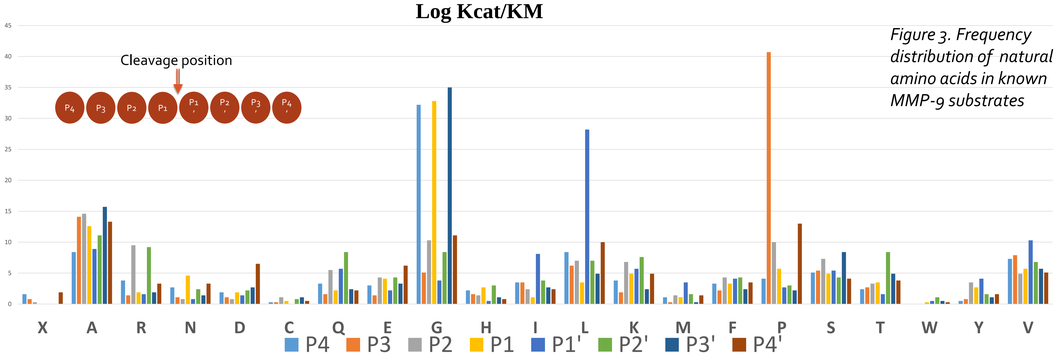


Figure 3. Frequency distribution of natural amino acids in known MMP-9 substrates

3. RESULTS:

- Y_scores were calculated as average of Lamort, Ratnikov and Kridel for literature known sequences (Table 1).
- Y_score against log Kcat/KM values of known peptides gave a polynomial regression curve with $R^2 = 0.9048$ (Figure 2).
- The frequency distribution of amino acids in natural materials that are responsive to MMP-9 is shown in Figure 3. This identifies key features of known substrates.
- Figure 4 shows how a good substrate for MMP-9 is predicted to be accommodated in the enzyme active site. This provides an explanation for many of the preferences shown in Figure 3.
- Table 2 presents a list of novel MMP-9 cleavable peptides with predicted Y_score and Kcat/KM. These are from a database of 1.2 million MMP-9 cleavable peptides that were generated by applying the above statistical and molecular modelling techniques.
- TEM image of self-assembled nano-micelle with MMP-9 cleavable peptide and a short rabies virus glycoprotein (sRVG) shuttle peptide (Figure 5). The size of nano-micelles ranged from 50-200 nm.

Table 2. Novel MMP-9 cleavable peptides with predicted cleavability (Kcat/KM)

Sequence	Y_score	Kcat/KM
PFM-AG	2.59	1,462,495
PFR-AG	2.51	1,264,381
PFR-LG	2.04	405,685
PKR-LG	2.03	389,159
PFR-FG	2.00	345,901

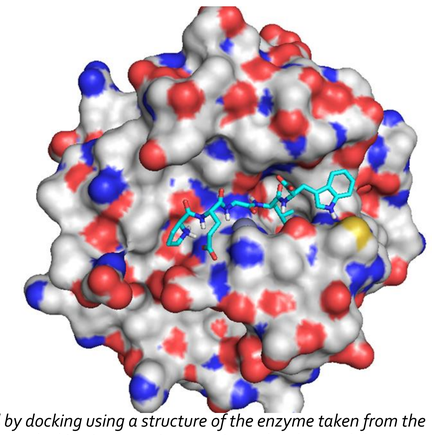
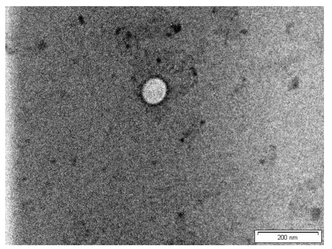


Figure 4. Characterising suitable biomaterial by docking using a structure of the enzyme taken from the protein databank. A peptide (PEGLW) after autodocking is shown in cyan; it can be observed that "Leu" extends into the S1' pocket and "Trp" wraps around the bridge over the S1' pocket.

Figure 5. TEM image of MMP-9 nano-micelle with targeting ligand



4. CONCLUSIONS:

- Novel MMP-9 cleavable peptides were generated by applying computational methods.
- Self assembled nano-micelles were formulated with MMP-9 cleavable and brain targeting peptides.

REFERENCES:

1. Kumar, P., Wu, H., McBride, J.L., Jung, K.E., Kim, M.H., Davidson, B.L., Lee, S.K., Shankar, P. and Manjunath, N., 2007. Transvascular delivery of small interfering RNA to the central nervous system. *Nature*, 448(7149), pp.39-43.
2. Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S. and Wood, M.J., 2011. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature biotechnology*, *29*(4), pp.341-345.
3. Javed, H., Menon, S.A., Al-Mansoori, K.M., Al-Wandi, A., Majbour, N.K., Ardah, M.T., Varghese, S., Vaikath, N.N., Haque, M.E., Azzouz, M. and El-Agnaf, O.M., 2015. Development of nonviral vectors targeting the brain as a therapeutic approach for Parkinson's disease and other brain disorders. *Molecular Therapy*.