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Peptides stapling as a tool for improving molecular recognition of C-reactive protein

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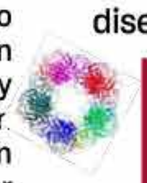
In our previous study, we identified several new CRP binding peptides (P2, P3, P9) using experimental (biological and physicochemical) methods supported by theoretical simulations (computational modelling analysis) [1]. The linear P3 peptide was successfully applied as a sensing element in a microfluidic sensing device for CRP recognition [2]. However, we are aware that linear peptides have some limitations, e.g. conformational instability. Thus, we have applied the stapling approach to solve that problem. We started with an in silico analysis to evaluate which of the studied peptides retain their recognition activity towards CRP. The calculations performed showed that the P9 peptide with a staple has a significantly higher affinity for CRP than linear peptides without a staple. The calculated binding energy for this stapled P9 peptide, which was lower than the values obtained for the linear form of the peptides (P2, P3, P9) or the stapled P2 and P3. The ring-closing olefin metathesis reaction was used to obtain the stapled P9 peptide. The new stapled peptide differs structurally from the linear form what was demonstrated using transmission electron microscopy and circular dichroism. The stapled P9 peptide was used for CRP recognition on silicate-modified indium tin oxide-coated glass electrodes. The obtained electrodes exhibit a better affinity towards CRP than electrodes modified with the linear form of this peptide. Moreover, the conformational stability of the P9 stapled peptide was also evaluated in the presence of proteases using CD and electrochemical studies.

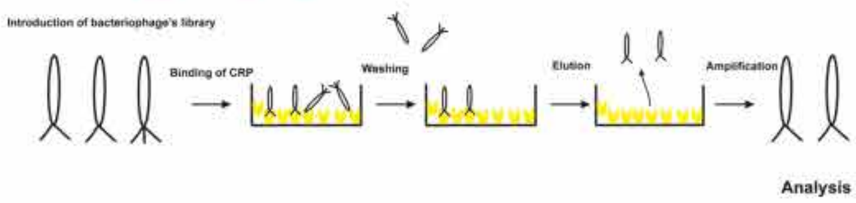
GOAL

To identify new receptors (peptides) for various disease markers and characterize their interactions



CRP - marker of inflammatory processes in human body
>10 mg/L inflammation
10-30 mg/L viral infection
>30 mg/L bacterial infection
Higher in cardiovascular, chronic and autoimmune diseases, tumors and COVID19

Phage dispaly - CRP clones selection



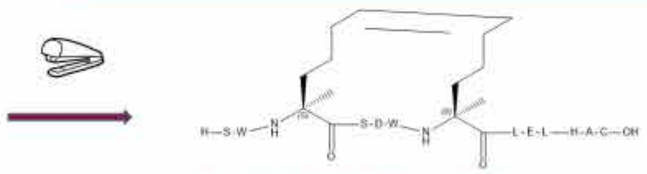
Stapling reaction

Ring-closing olefin metathesis [3]

SWFSDWDLELHA
P9 peptide

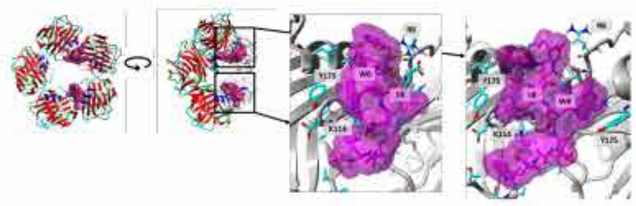
Amino acids sequence analysis, synthesis of selected peptides

CRP peptides	Amino acids sequence											
P2	G	G	S	D	P	E	G	M	Q	G	N	Y
P3	V	H	W	D	F	R	Q	W	W	Q	P	S
P9	S	W	F	S	D	W	D	L	E	L	H	A



Stapled (St.) P9 peptide

Molecular docking analysis



The binding energy of selected peptides in complex with CRP.

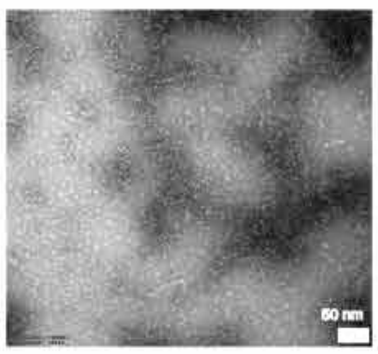
CRP- P9 peptide -51.7 kcal/mol

CRP- St. P9 peptide -79.1 kcal/mol

Transmisson electron microscopy analysis

P9 peptide

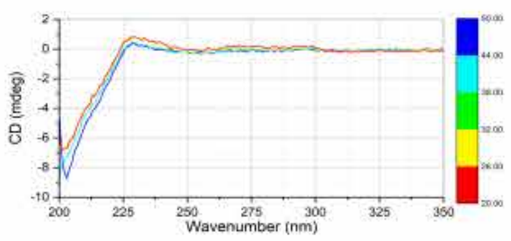
Stapled P9 peptide

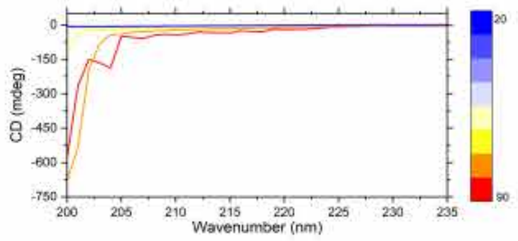


Circular dichroism analysis

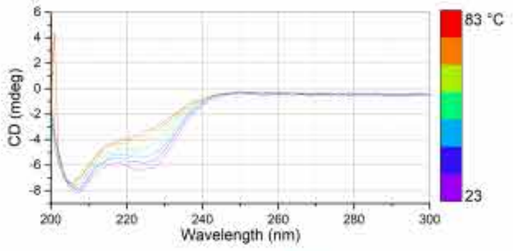
Temperature stability

P9 peptide



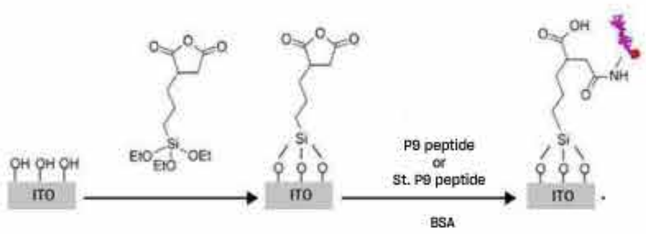


Stapled P9 peptide



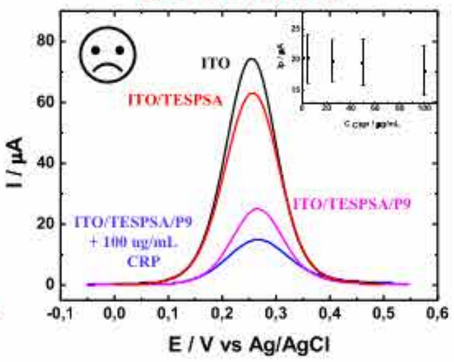
Electrochemical studies of CRP recognition by P9 peptide and St. P9 peptide

Schematic illustration of ITO electrode modification

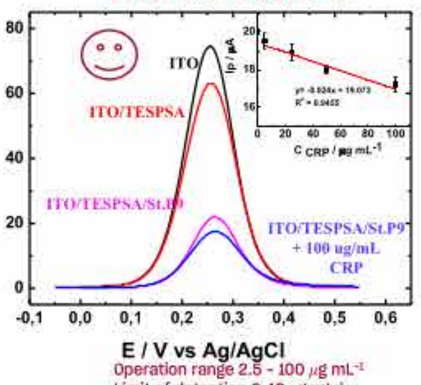


1mM 1,1'-ferrocenodimethanol/PBS

ITO/TESPSA/P9

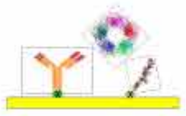


ITO/TESPSA/St. P9



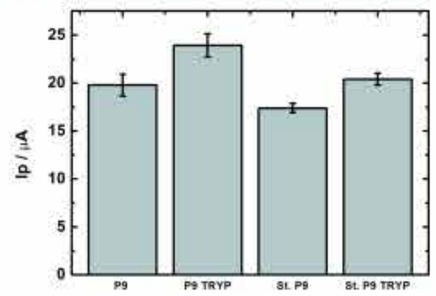
Operation range 2.5 - 100 $\mu g\ mL^{-1}$
Limit of detection 0.46 $\mu g\ mL^{-1}$

10-30 mg/L - VIRUS



> 30 mg/L - BACTERIA

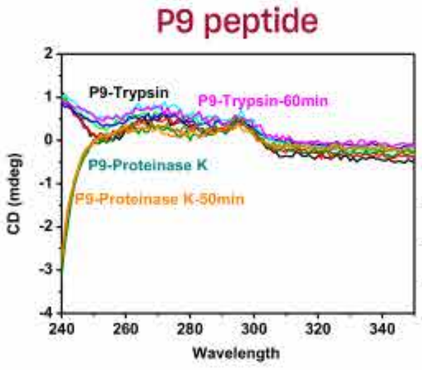
Effect of trypsin on electrochemical activity towards CRP



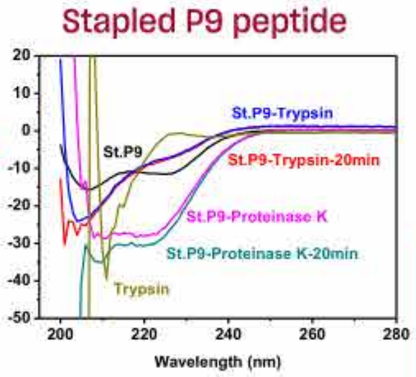
CRP (2.5 $\mu g\ mL^{-1}$)
Trypsin - TRYP (200 ng mL^{-1})

Circular dichroism analysis

P9 peptide



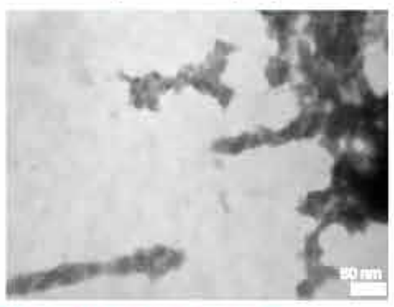
Stapled P9 peptide



After treatment with proteases

P9 peptide

Stapled P9 peptide



Conclusions

Three CRP binding peptides were found based on the phages display technique.

The result shows that the stapled P9 peptide exhibits higher affinity toward CRP than linear P9.

The conformational structure of the stapled P9 peptide is more resistant to external factors (temperature, protease activity) than linear P9 peptide.

The stapled P9-based electrode exhibits response towards the CRP in a wide range of concentration and in the presance of proteases.

It is possible to use P3 peptide to distinguish between virial and bacterial infection.

References:

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Acknowledgments

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