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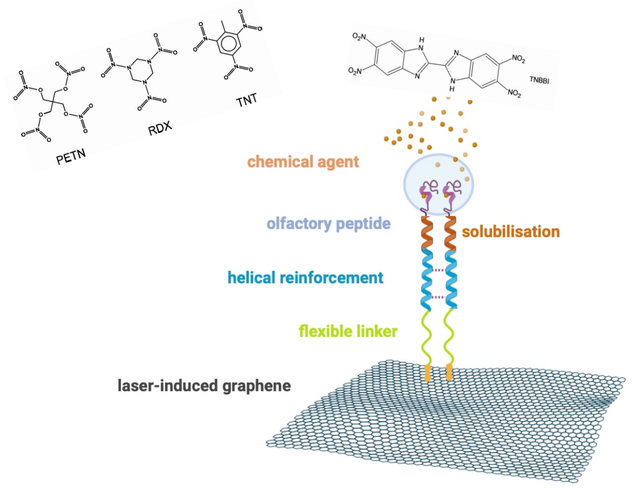
Olfactory Inspired Peptides as Tunable Platforms for Selective Detection of Explosives

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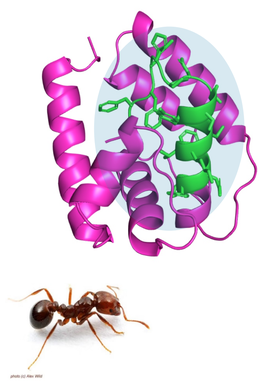
Motivation

Olfactory systems have inspired **biosensor** design due to their ability to fine-tune interactions between amino acid sequences and target volatile compounds for selectivity and sensitivity[1]. In this project's first phase, **insect-derived** olfactory receptors and odorant-binding proteins were used to design **artificial peptides** sensitive to **TNT** and **DNT.** These peptides were synthesized using solid-phase peptide synthesis and characterized by solution NMR spectroscopy. MD simulations assessed their interactions with TNT/DNT, aiding peptide optimization. Future work includes chemical shift mapping of these interactions and immobilizing peptides on **laser-induced graphene** substrates for cost-effective electrochemical sensors targeting **chemical warfare** agents.



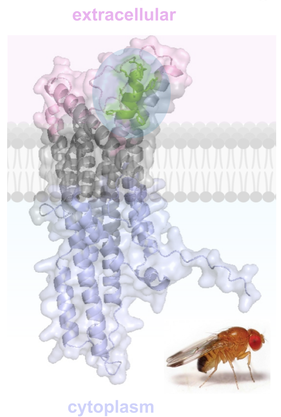
Architecture of the peptide portion of the biosensor. Individual functional parts are highlighted.

siOBP5 from *Solenopsis invicta*



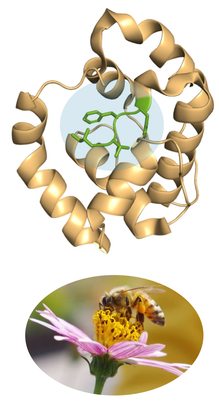
C-terminus from Odorant-binding protein (OBP)
TYQFSKGSYSTDPDHFF
binds alarm pheromone[2]

Or67a from *Drosophila melanogaster*



Soluble helix from Odorant receptor (OR)
SWLFYPSYFH
general activity towards aromatic compounds[3]

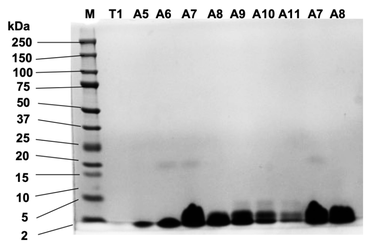
ASP1 from *Apis mellifera*



Pheromone-binding site of Odorant-binding protein (OBP)
WFVI
TNT-reactive[4]

Name	Sequence	No. of AA
P4	GGGGLQELNQKLGYFVIG	18
P5	GGGGLQELTSKLGWFVIG	18
P6	CGGGGLQELTSKLGWFVIG	19
P8	GGGGLQELTSKLHANFSKYILHQRG	25
P9	GGGGLQELTSKLKARGSAEGWFVIG	25
P10	GGGGLQELTSKLKARGSAEGSWLKYPSYFHG	31
P11	GGGGLQELTSKLKARGSAEGSWLKPSFHG	29
P12	GGGGKARGSAEGTYQFSKGSYSTDPDHFFG	30
P13	GGGGKARGSAEGTYQFSKGSYSTHFG	26
P14	GGGGKARGSAEGWHSAMAKGVYG	23

Polyglycine…flexibility
Solubilisation insert
Helicity-inducing region
Sensing region



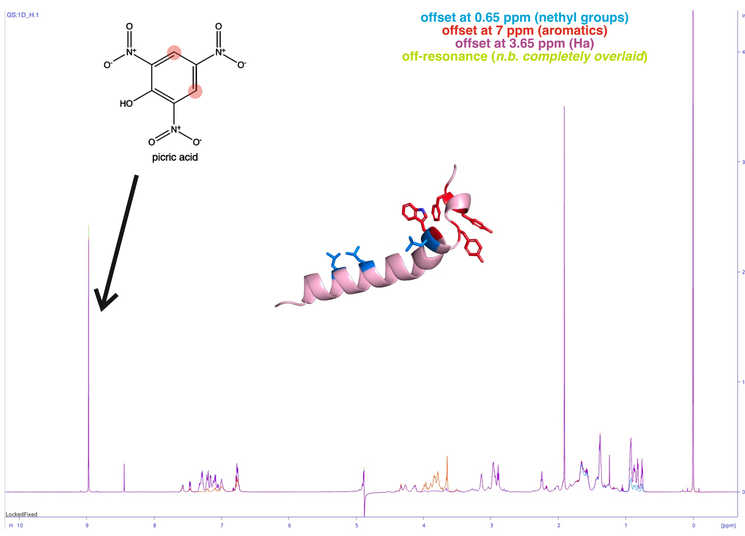
Tris-Tricine SDS-PAGE

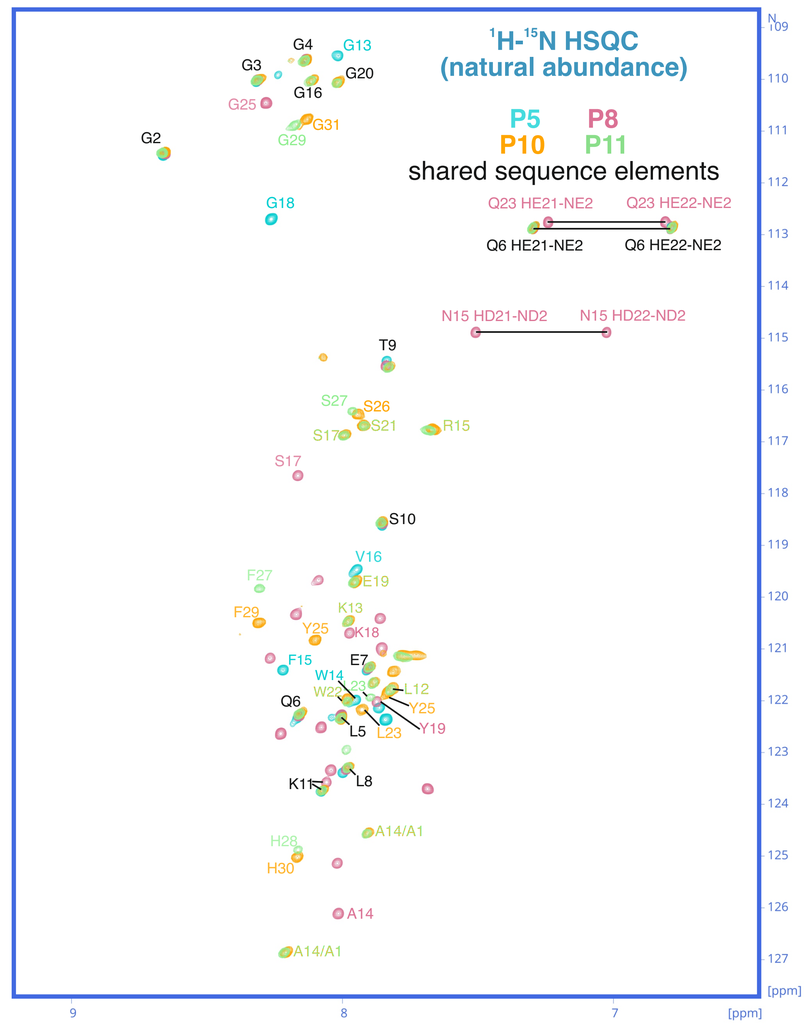
Solid-phase peptide synthesis

Peptides were synthesized on a 0.1 mmol scale using Wang resin and an automated microwave-assisted SPPS synthesizer (Liberty Blue 1.0, CEM). Fmoc-protected amino acids with appropriate side-chain protection were used. Cleavage from resin was performed via TFA, and products were precipitated with diethyl ether. Yields ranged from 55–91% (100–350 mg crude product, depending on peptide size). Peptides were purified alternatively by ion-exchange chromatography (HiTrap CaptoS/Q, Cytiva) or HPLC (70% acetonitrile gradient) and analyzed by Tris-Tricine SDS-PAGE. Final product quality was confirmed by HPLC ESI-MS (0.1% formic acid/acetonitrile gradient; Agilent TOF/Q-TOF).

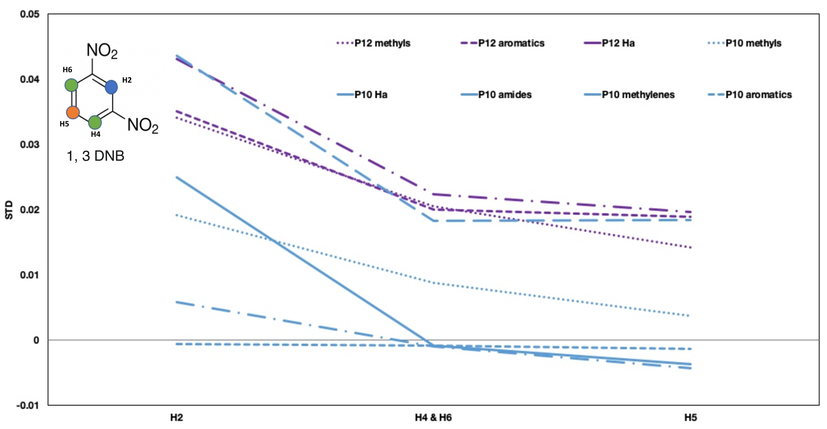
NMR spectroscopy

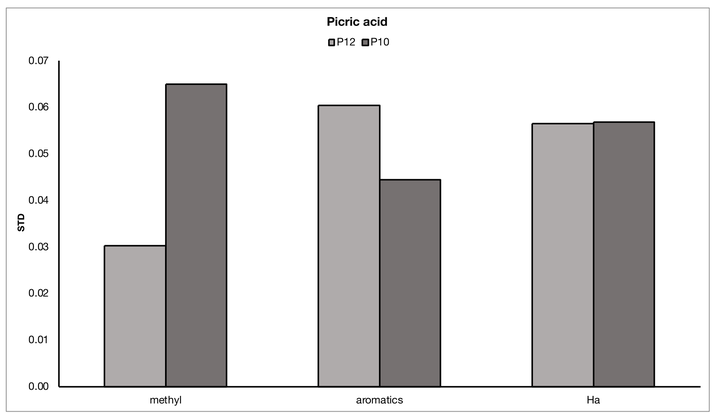
Peptides were analyzed as crude products in **DMSO-d$_6$** at 298 K on a Bruker Ascend **700 MHz** with TCI cryoprobe, at 30–40 mM concentration and **natural isotope abundance**. Due to sequence repetition and DMSO-induced unfolding, standard 2D methods (TOCSY, COSY, HMBC/HMQC) were ineffective. Instead, a strategy using ^{1}H-^{15}N HSQC, ^{1}H-^{13}C HSQC, ^{1}H-^{15}H SQC-TOCSY, and ROESY was employed. Full **assignment spectra** for one peptide required **16–20 hours**; 2D HSQC-TOCSY was acquired with **50% non-uniform sampling** (NUS). **Saturation transfer difference** NMR was probed to observe interactions between surrogate ligands (1, 3 dinitrobenze, picric acid) and peptides in D_2O.



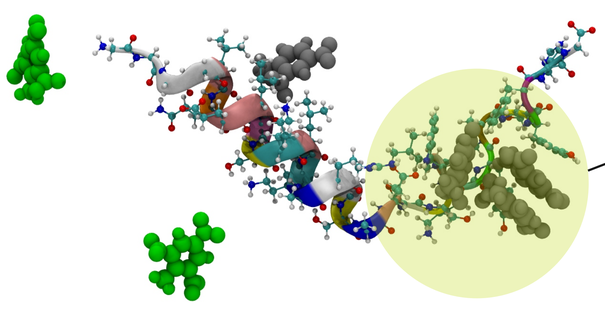


STD shows selectivity of P10 and P12 towards picric acid and 1,3 DNB



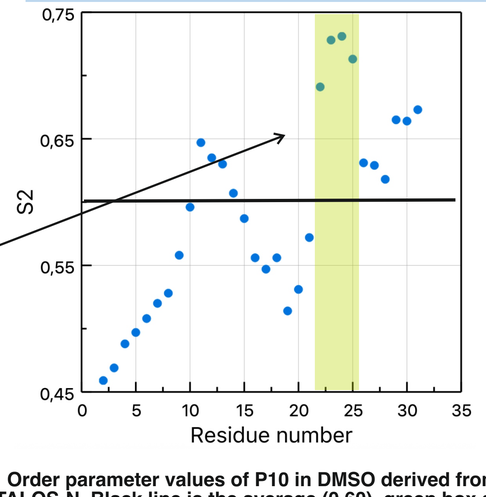


DNT binding to P10 obtained by MD simulations



Result of P10 interaction (AF^2 model) with 4 DNT and 4 TNT molecules W22 and Y25/Y28 in detail with 3 DNT molecules

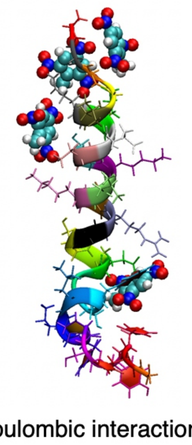
Predicted binding site most rigid in DMSO



Order parameter values of P10 in DMSO derived from TALOS-N. Black line is the average (0.60), green box are residues W22-Y25

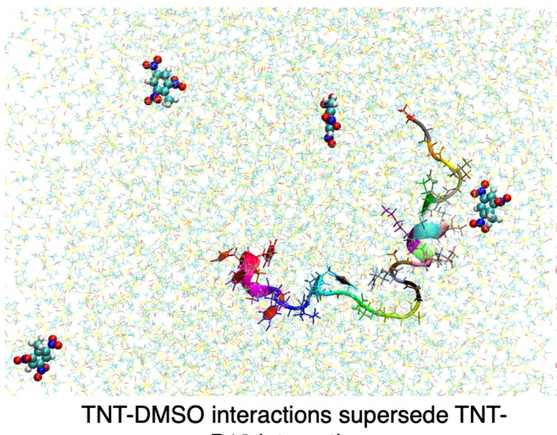
Role of a polar-solvent essential for TNT and DNT interactions with peptides

Gas-phase



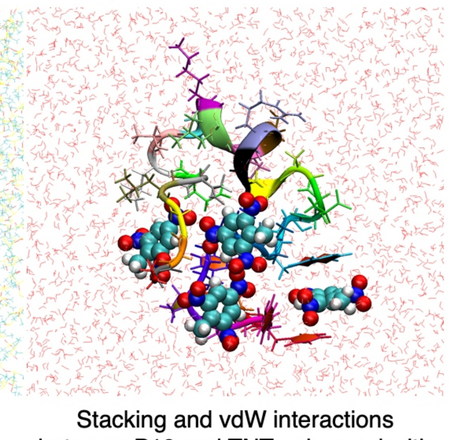
Coulombic interactions

Gas-phase structure solvated in DMSO



TNT-DMSO interactions supersede TNT-P10 interactions

Gas-phase structure solvated in water



Stacking and vdW interactions between P10 and TNT enhanced with water environment

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