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Identification of reactivity hotspots at the interfaces of protein–protein interactions

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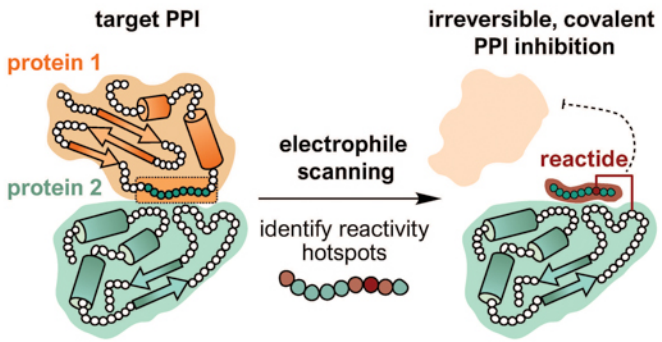
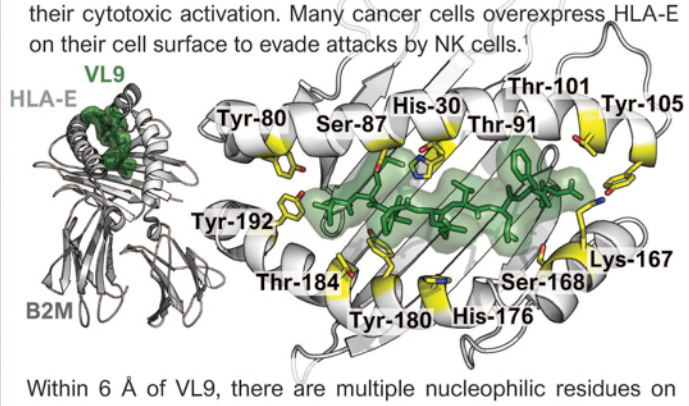
RATIONALE



Covalent binding can enhance the activity of drugs by prolonged target engagement and increased selectivity. Peptides, which are prone to rapid degradation and elimination, may benefit from this approach. However, selecting a residue for covalent binding is challenging due to the effects of the local microenvironment. We propose *electrophile scanning* to identify reactivity hotspots in peptide ligands to covalently bind and disrupt disease-related protein–protein interactions (PPIs).

TARGET

VL9, a 9-mer peptide antigen presented by HLA-E on healthy cells was subjected to an electrophile scan. The complex is recognized CD94–NKG2A on natural killer (NK) cells to prevent their cytotoxic activation. Many cancer cells overexpress HLA-E on their cell surface to evade attacks by NK cells.[1]

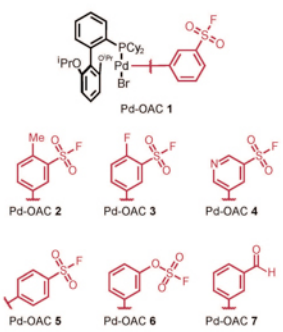


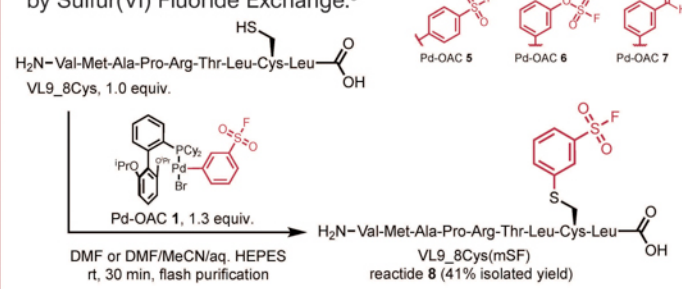
Within 6 Å of VL9, there are multiple nucleophilic residues on HLA-E for covalent binding with protein-reactive modifier.

1) Borst et al. *Clinical Cancer Research* **2020**, *26* (21), 5549-5555

REACTIDE SYNTHESIS

Unprotected, crude analogs of VL9 were equipped with versatile meta-sulfonyl fluoride (mSF) in one step. A palladium oxidative addition complex (Pd-OAC) introduced the electrophilic group on Cys-modified analogs of VL9.[2] mSF was used for initial studies due to broad reactivity by Sulfur(VI) Fluoride Exchange.[3]

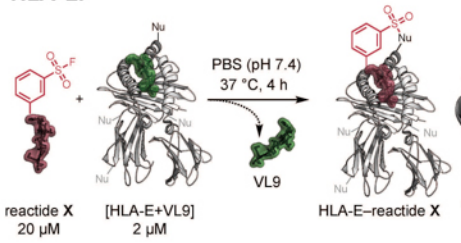


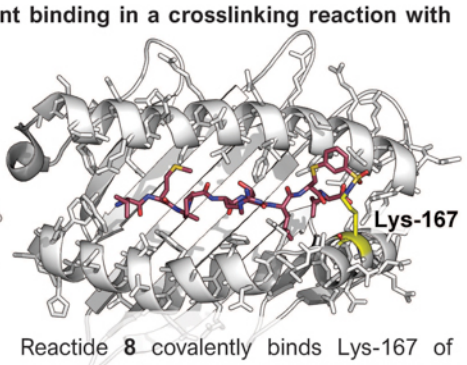


2) Narayanan et al. *Chemical Science* **2015**, *6* (5), 2650-2659
3) Vinogradova et al. *Nature* **2015**, *526* (7575), 687-691

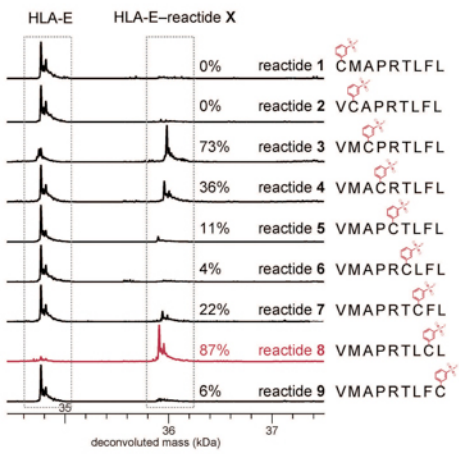
CROSSLINKING RESULTS

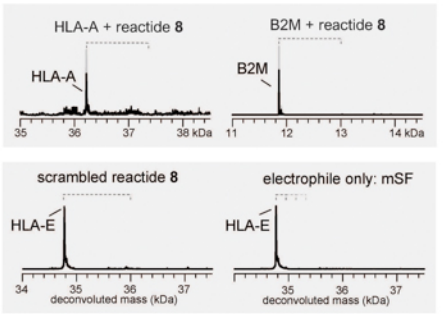
Several reactides showed potent covalent binding in a crosslinking reaction with HLA-E.



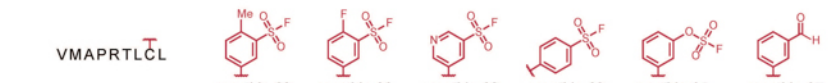


Reactide **8** covalently binds Lys-167 of HLA-E as determined by MS² analysis of the conjugate. No binding was found with off-target proteins, and reactide controls.



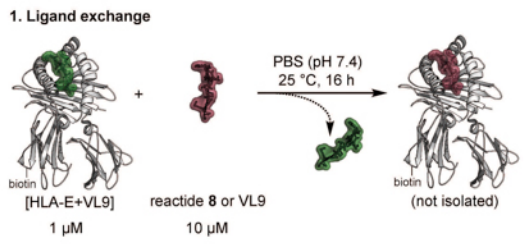


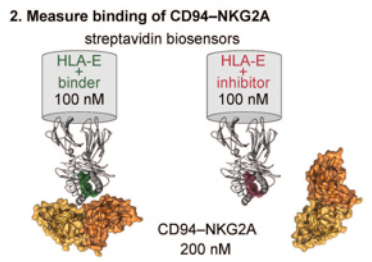
Pd-OACs **2–7** were used to explore the crosslinking potential of diverse electrophilic groups on VL9_8Cys. The readily available, shelf-stable Pd-OACs allow for further optimization of reactivity and stability profiles at reactivity hotspots.



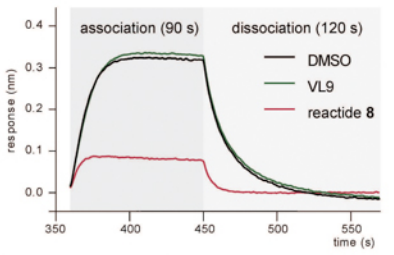
	reactide **10**	reactide **11**	reactide **12**	reactide **13**	reactide **14**	reactide **15**
crosslinked HLA-E after 4 h:	69%	82%	74%	17%	n. obs	n. obs

PPI INHIBITION





Reactide 8 inhibits the PPI of HLA-E and CD94–NKG2A. After ligand exchange in the antigen-binding groove of HLA-E and covalent binding by reactide **8**, the receptor heterodimer of NK cells showed reduced binding to HLA-E. Inhibiting the molecular recognition of cancer cells by NK cells could be a useful intervention in cancer immunotherapy.



CONCLUSION

Peptides with covalent modes of action are a promising modality for the modulation of therapeutically relevant PPIs. Electrophile scanning identifies reactivity hotspots at early stages of ligand discovery and development and provides targeting options to guide further maturation efforts.

Full paper:
Grob NM, et al. *ACS Chem Bio* **2024**, *19* (1), 101-109

Current activities:
Grob Group for Peptide-based Drug Discovery at ETH zürich

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