Exploring Motif Preferences and Functional Specificity of the Protein Arginine Deiminase Family

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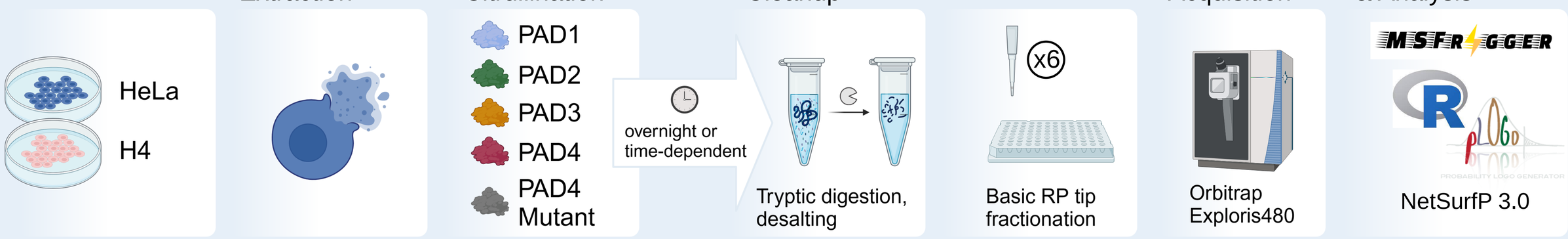
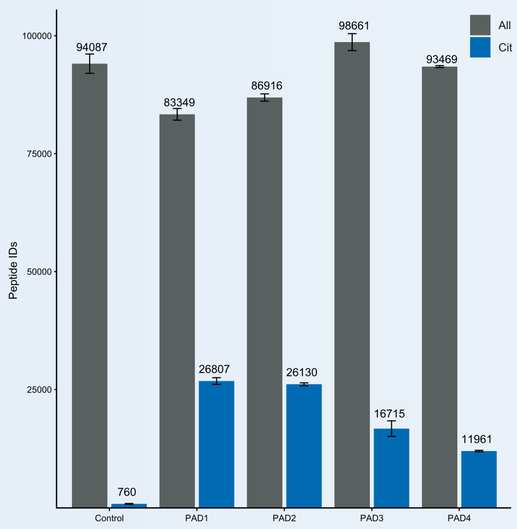
Experimental Design

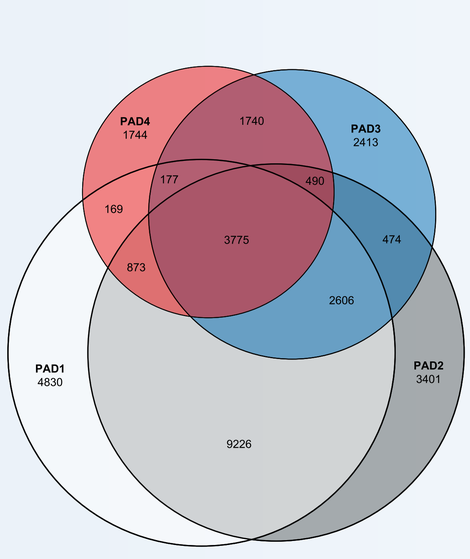


Different PAD Enzymes

Peptide IDs

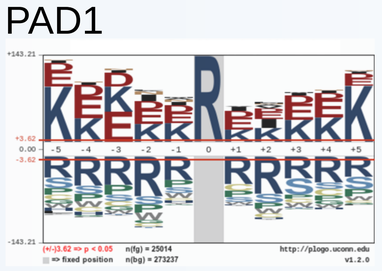


Citrullination Sites



Motif Analysis

PAD1



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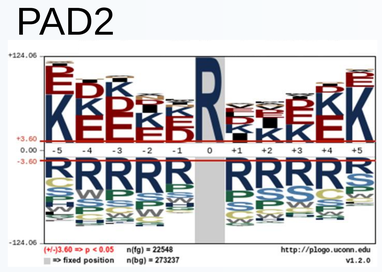
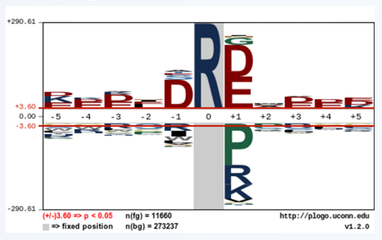
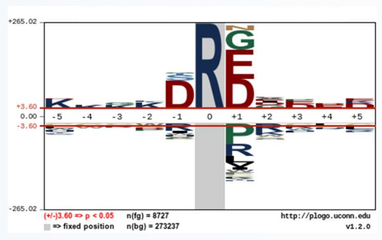


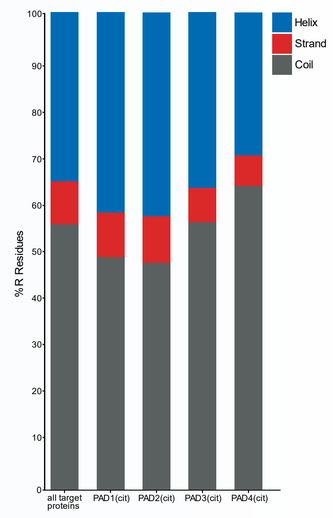
PAD3



PAD4

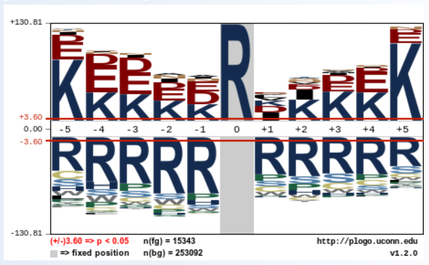


Predicted Structure

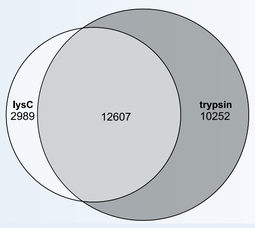


Lys-C Digestion

Lys-C Peptide Motif

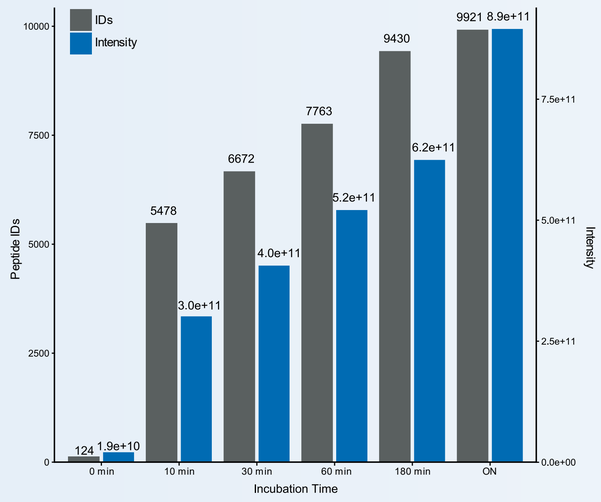


Lys-C vs Trypsin Sites



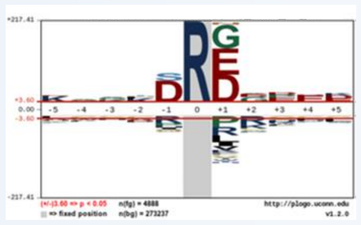
Time-dependent Analysis of PAD4

Peptide IDs & Intensities

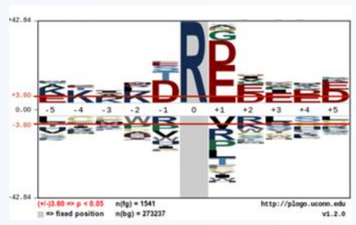


New sites per timepoint

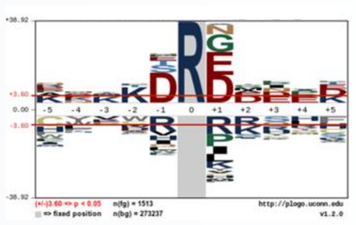
10min



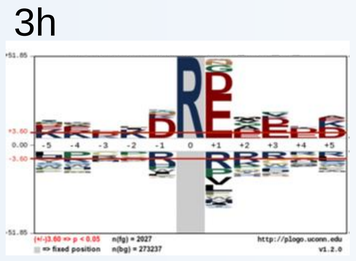
30min



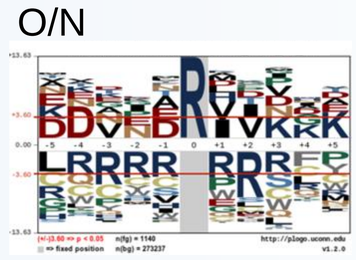
1h



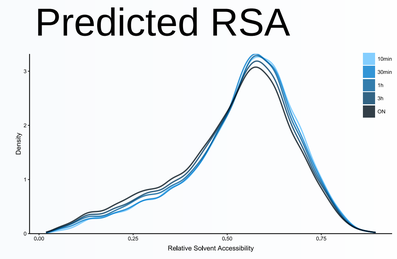
3h



O/N

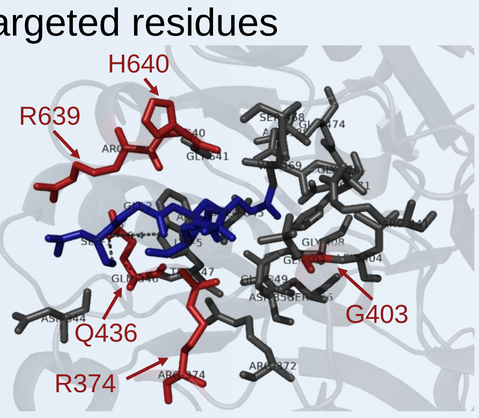


Predicted RSA



PAD4 Mutagenesis

Targeted residues



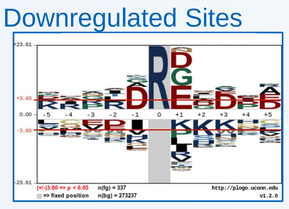

```
PAD1| DSPRNRGLKDFPYKRILGPDFGYVTREIPLPGPSSLDSFGNL
PAD2| DSPRDGNLKDFPVKELLGPDFGYVTREPLFESVTSLDSFGNL
PAD3| DSPRNGELQDFPYKRILGPDFGYVTREPRDRSVSGLDSFGNL
PAD4| DSPRNRGLKEFPIKRVMGPDFGYVTRGPQTGGISGLDSFGNL
          374                              403
```

Kinetic Parameters

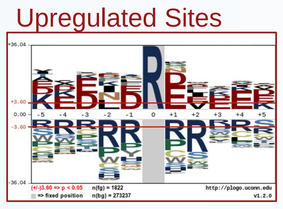
	K_m (mM)	k_{cat} (s^{-1})	K_{cat}/K_m
PAD4	0.64±0.06	5.90±0.16	9.27±2.80
Q346R	0.86±0.05	3.86±0.06	4.49±1.46
R374G	0.27±0.01	4.99±0.04	18.30±4.39
G403S	0.40±0.03	5.67±0.13	14.38±3.83
R639F	0.20±0.03	5.48±0.15	26.95±5.90
H640L	0.33±0.04	5.32±0.16	16.10±4.09
Q346R|G403S	0.17 ± 0.01	3.37 ± 0.05	19.82 ± 5.00
Q346R|R639F	0.12 ± 0.04	3.25 ± 0.18	27.08 ± 4.50
G403S|H640L	0.16 ± 0.02	7.07 ± 0.16	44.18 ± 8.00
R639F|H640L	0.06 ± 0.04	2.39 ± 0.18	39.83 ± 4.50
Q346R|R639F|H640L	0.02 ± 0.01	2.36 ± 0.04	118.00 ± 4.00
Q346R|G403S|R639F|H640L	0.02 ± 0.00	3.04 ± 0.01	152.00 ± 0.00

Single mutation at Q346

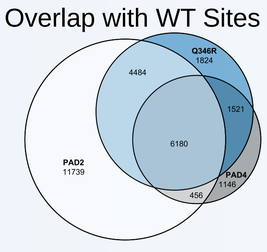
Downregulated Sites



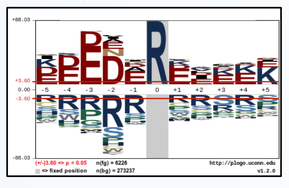
Upregulated Sites



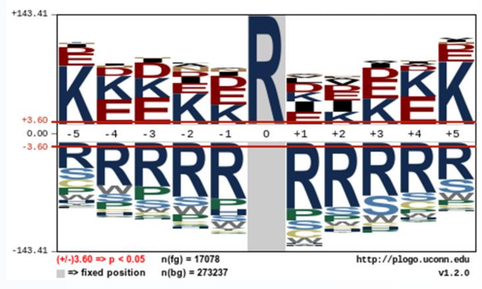
Overlap with WT Sites



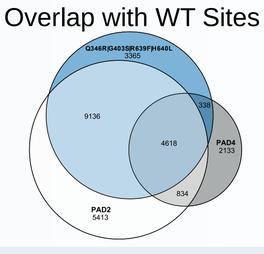
New Sites



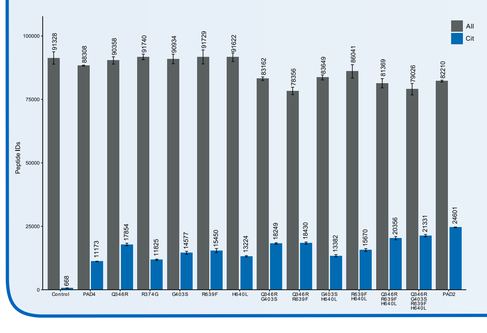
Quadruple Mutation



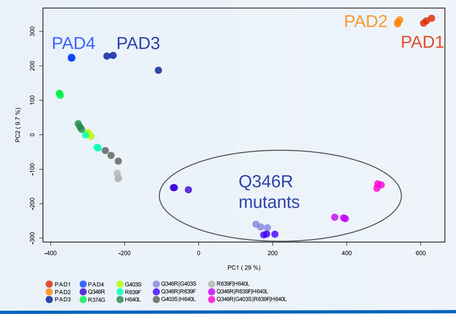
Overlap with WT Sites



Peptide IDs



PCA of WT and Mutants



Conclusions

- PAD isozymes show distinct target preferences on protein and sequence level
- Established PAD4 substrate motif is consistent throughout increasing incubation times and independent of protease
- Residues involved in substrate selection of PAD4 are identified
- Mutations of individual residues impacts substrate selection of PAD4

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