Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Modulating Substrate Specificity of Histone Acetyltransferase with Unnatural Amino Acids

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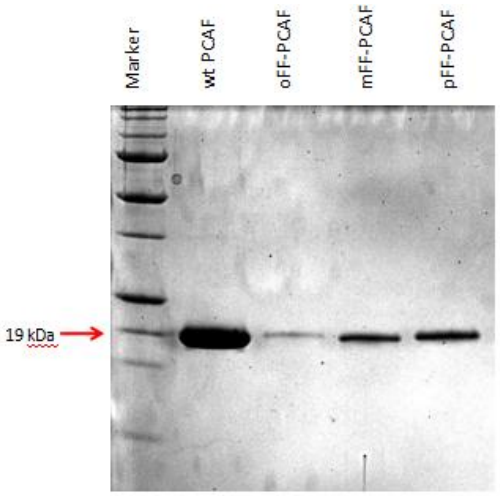

Figure S1a. SDS-PAGE gel of PCAF purification with the lanes illustrated.

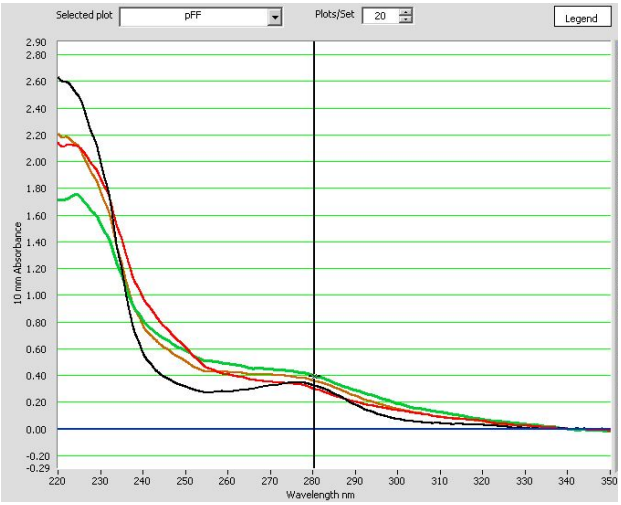

Figure S1b. UV-vis spectra (cursor at 280 nm) of wt-PCAF (black), oFF-PCAF (red), mFF-PCAF (orange) and pFF-PCAF (green).

Hitachi L8900 Amino Acid Analyzer Report

Analyzed: 3/2/2011 11:06:49 PM Reported: 3/4/2011 2:50:40 PM
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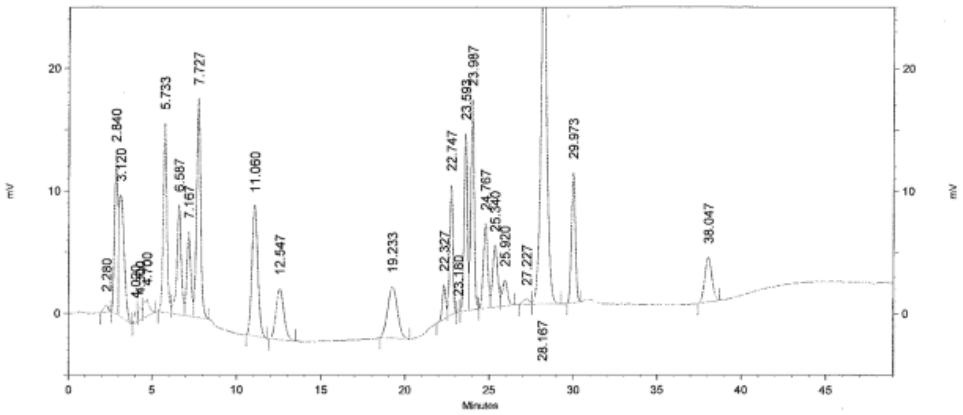
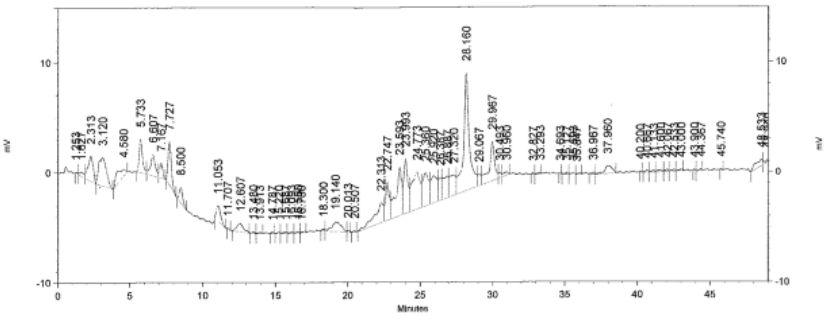
Sample ID: Mehta PCAF
Vial Number: 156 Inj. Volume(uL): 50 Loading buffer volume (ul): 200



VIS 1 Results

Pk #	RT	Name	ESTD concentration	Area	Height	Current RF
		Cysac	0.0000 BDL			
1	2.280		0.0000	43673	2309	
2	2.840		0.0000	704058	51843	
3	3.120	Int. Std	2.7731	866943	39883	0.0000017652
4	4.020		0.0000	44043	3497	
5	4.300		0.0000	63175	4000	
6	4.700	CMCys	0.0000	133040	5660	0.0000000000
7	5.733	Asp	0.4697	944768	62000	0.0000004971
8	6.587	Thr	0.3034	650824	35854	0.0000004662
9	7.167	Ser	0.2018	442880	27501	0.0000004557
10	7.727	Glu	0.5558	1177208	71751	0.0000004721
11	11.060	Gly	0.5043	1015762	42673	0.0000004965
12	12.547	Ala	0.2879	551220	16753	0.0000005224
		Cys	0.0000 BDL			
13	19.233	Val	0.3451	676330	16916	0.0000005103
14	22.327	Met	0.0812	162672	11179	0.0000004989
15	22.747	nVal	0.2380	542660	42662	0.0000004387
16	23.180		0.0000	6202	779	0.0000006063
17	23.593	Ile	0.4014	764058	57949	0.0000005253
18	23.987	Leu	0.4211	946916	68664	0.0000004447
19	24.767	Tyr	0.2427	503354	27626	0.0000004822
20	25.340	oFF	0.2412	397924	20149	
21	25.920	Phe	0.0862	175242	8413	0.0000004920
22	27.227		0.0000	46326	1742	
23	28.167	NH3	0.8034	3067166	131300	0.0000002619
24	29.973	His	0.3221	697310	42532	0.0000004620
		Trp	0.0000 BDL			
25	38.047	Arg	0.2028	433630	14563	0.0000004677



VIS 2 Results

Pk #	RT	Name	ESTD concentration	Area	Height	Current RF
10	8.500	Pro	0.2679	95069	5761	0.0000028184

Figure S2a. Amino acid analysis chromatogram for oFF-PCAF.

Hitachi L8900 Amino Acid Analyzer Report

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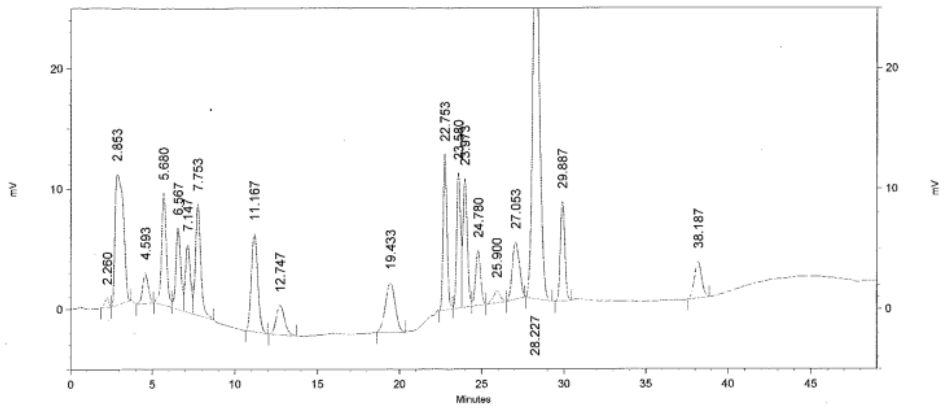
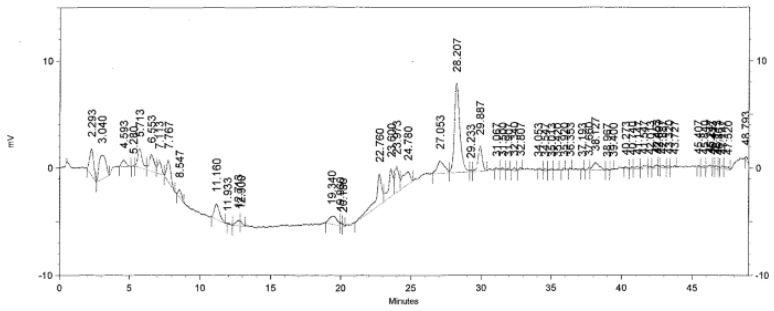
Sample ID: Mehta pFF
Vial Number: 158 Inj. Volume(uL): 50 Loading buffer volume (ul): 100



VIS 1 Results

Pk #	RT	Name	ESTD concentration	Area	Height	Current RF
		Cysac	0.0000 BDL			
1	2.260		0.0000	58934	3196	
2	2.853	Int. Std	2.5456	1497074	43483	0.0000017004
3	4.593	CMCys	0.0000	247177	10291	0.0000000000
4	5.680	Asp	0.3693	775301	37134	0.0000004764
5	6.567	Thr	0.2493	568579	27021	0.0000004384
6	7.147	Ser	0.2034	467227	22620	0.0000004353
7	7.753	Glu	0.3695	808268	36948	0.0000004572
8	11.167	Gly	0.4220	867572	32617	0.0000004864
9	12.747	Ala	0.1707	341480	9818	0.0000005000
		Cys	0.0000 BDL			
10	19.433	Val	0.3325	669760	16461	0.0000004964
		Met	0.0000 BDL			
11	22.753	nVal	0.3621	854175	51658	0.0000004239
12	23.580	Ile	0.3292	740215	44932	0.0000004447
13	23.973	Leu	0.3405	765044	42707	0.0000004450
14	24.780	Tyr	0.1736	388688	18164	0.0000004465
15	25.900	Phe	0.0529	114650	3971	0.0000004616
16	27.053	pFF	0.2299	565524	18984	
17	28.227	NH3	0.8902	3755808	127803	0.0000002370
18	29.887	His	0.2863	632283	32958	0.0000004529
		Trp	0.0000 BDL			
19	38.187	Arg	0.1773	378827	12105	0.0000004680



VIS 2 Results

Pk #	RT	Name	ESTD concentration	Area	Height	Current RF
9	8.547	Pro	0.1368	50907	3091	0.0000026871

Figure S2b. Amino acid analysis chromatogram for pFF-PCAF.

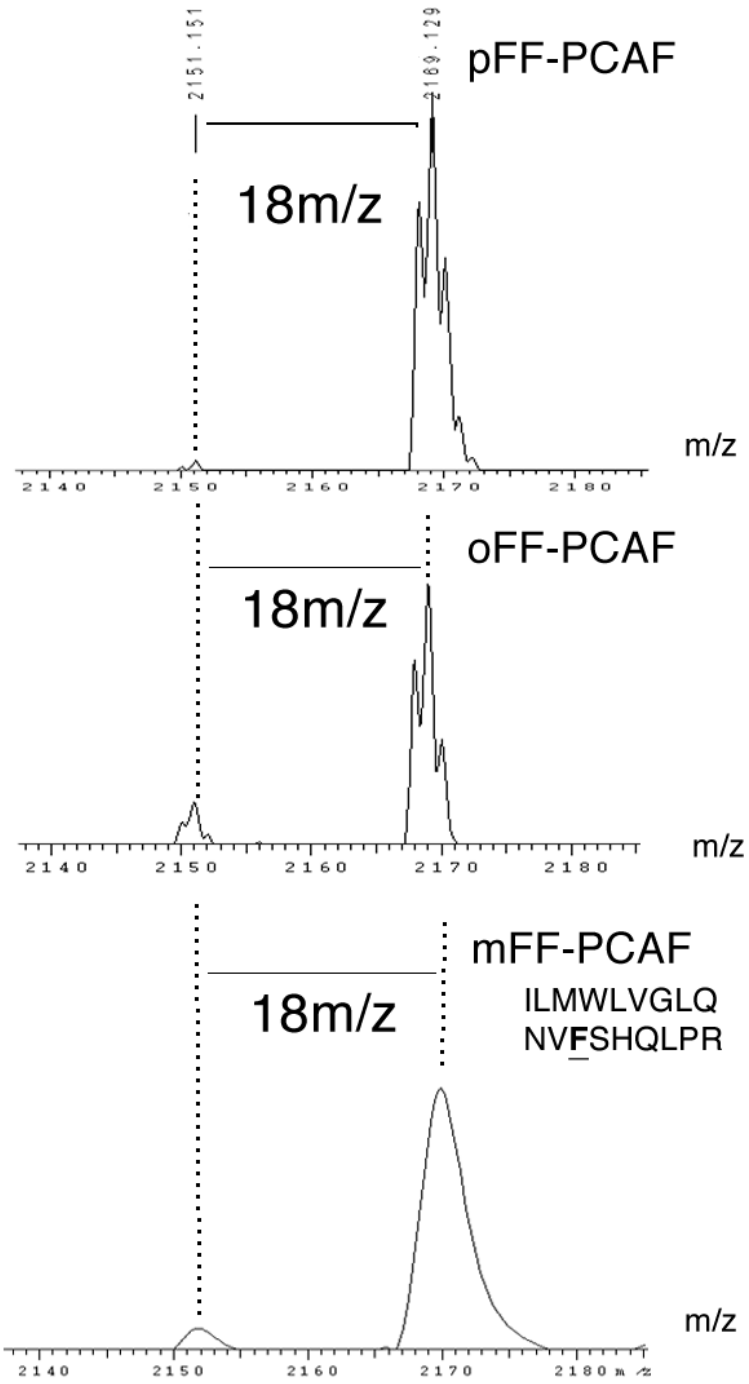


Figure S2c. Mass spectrometric analysis of oFF-PCAF, mFF-PCAF and pFF-PCAF after trypsin digestion demonstrating the mass peaks of trypsin peptide fragments.

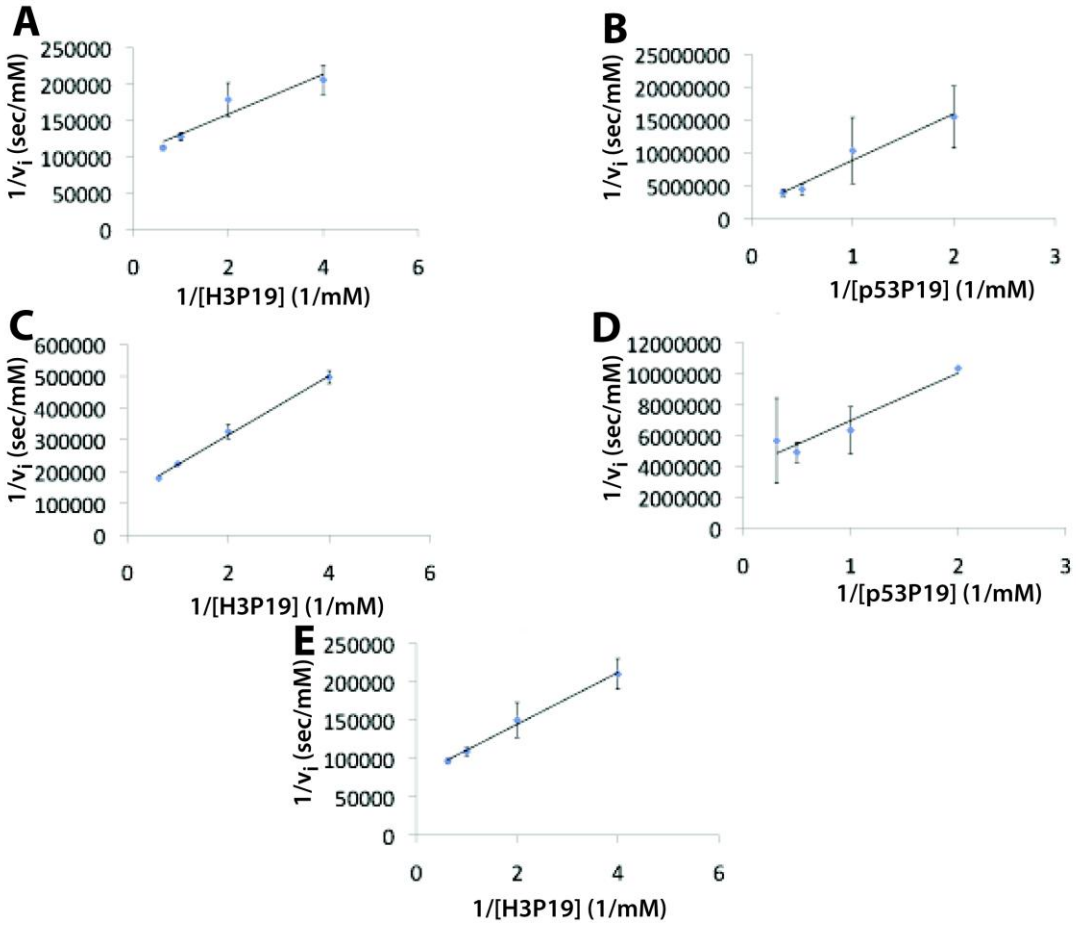


Figure S3. Line-Weaver Burk plots of Michelis Menten kinetics. (A) wt•H3p19 (B) wt•p53p19 (C) pFF-PCAF•H3p19 (D) pFF-PCAF•p53p19 (E) mFF-PCAF•H3p19.

H3P19 Peptide Sequence: QTARKSTGGKAPRKLASK
p53P19 Peptide Sequence: NTSSSPQPKKKPLDGEYFT

Figure S4. H3p19 and p53p19 peptide sequence.[1-2]

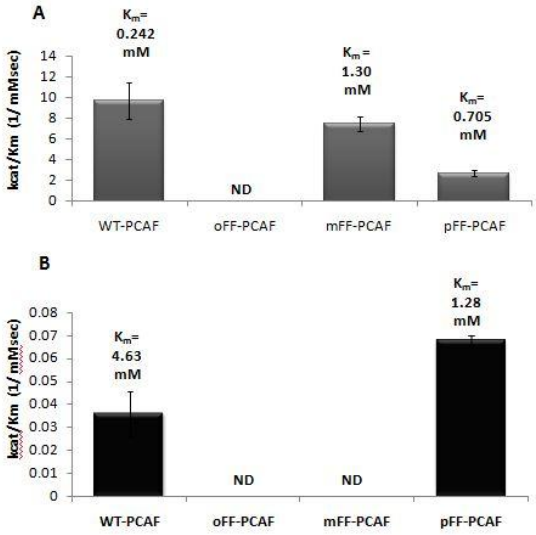


Figure S5. Michelis Menten kinetics parameters of wt and fluorinated PCAF with (A) H3p19 in grey and (B) p53p19 in black.

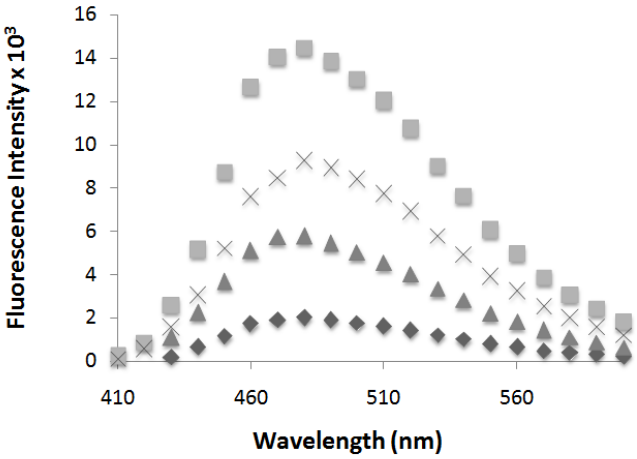


Figure S6. ANS binding of wt PCAF (diamonds), oFF-PCAF (squares), mFF-PCAF (triangles) and pFF-PCAF (crosses) in the presence of p53p19 and AcCoA.

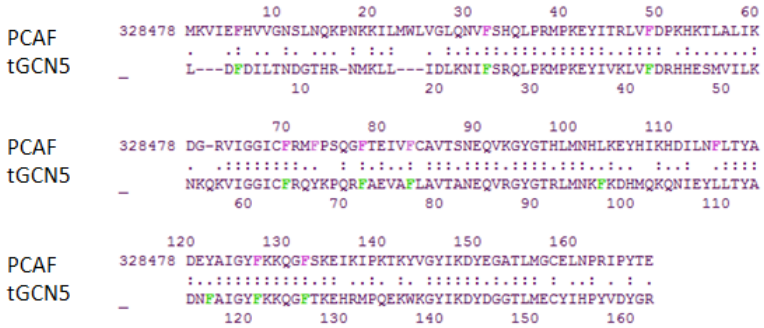


Figure S7a. Sequence alignment of the HAT domain of PCAF and tGCN5 with the phenylalanine residues highlighted in pink and green for PCAF and tGCN5 respectively.

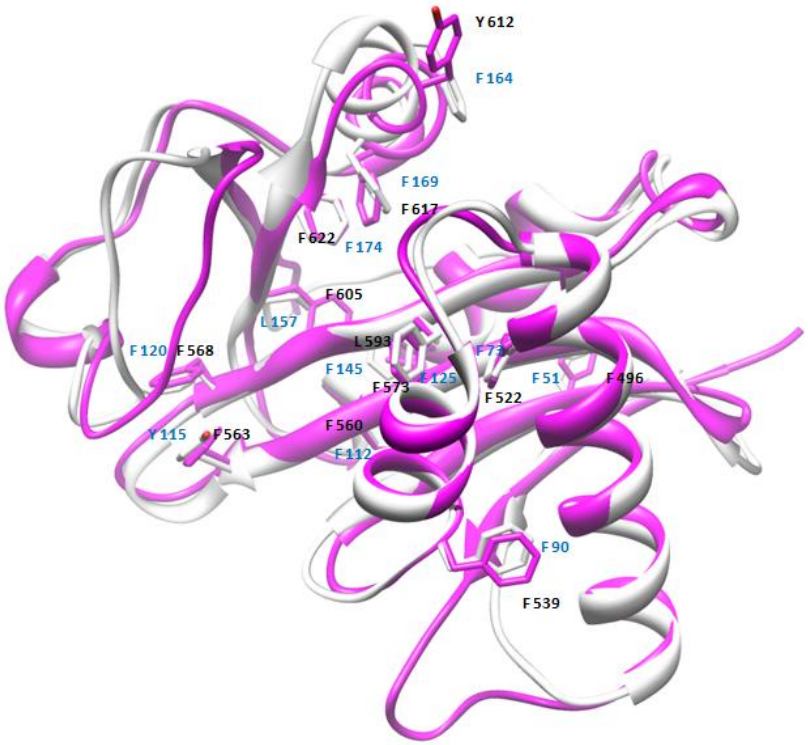


Figure S7b. Structural alignment of the crystal structures of tGCN5 (Grey ribbon) and PCAF (Pink ribbon). The phenylalanine residues and the corresponding homologous residues are labeled in both, tGCN5 (blue) and PCAF (black).[3-4]

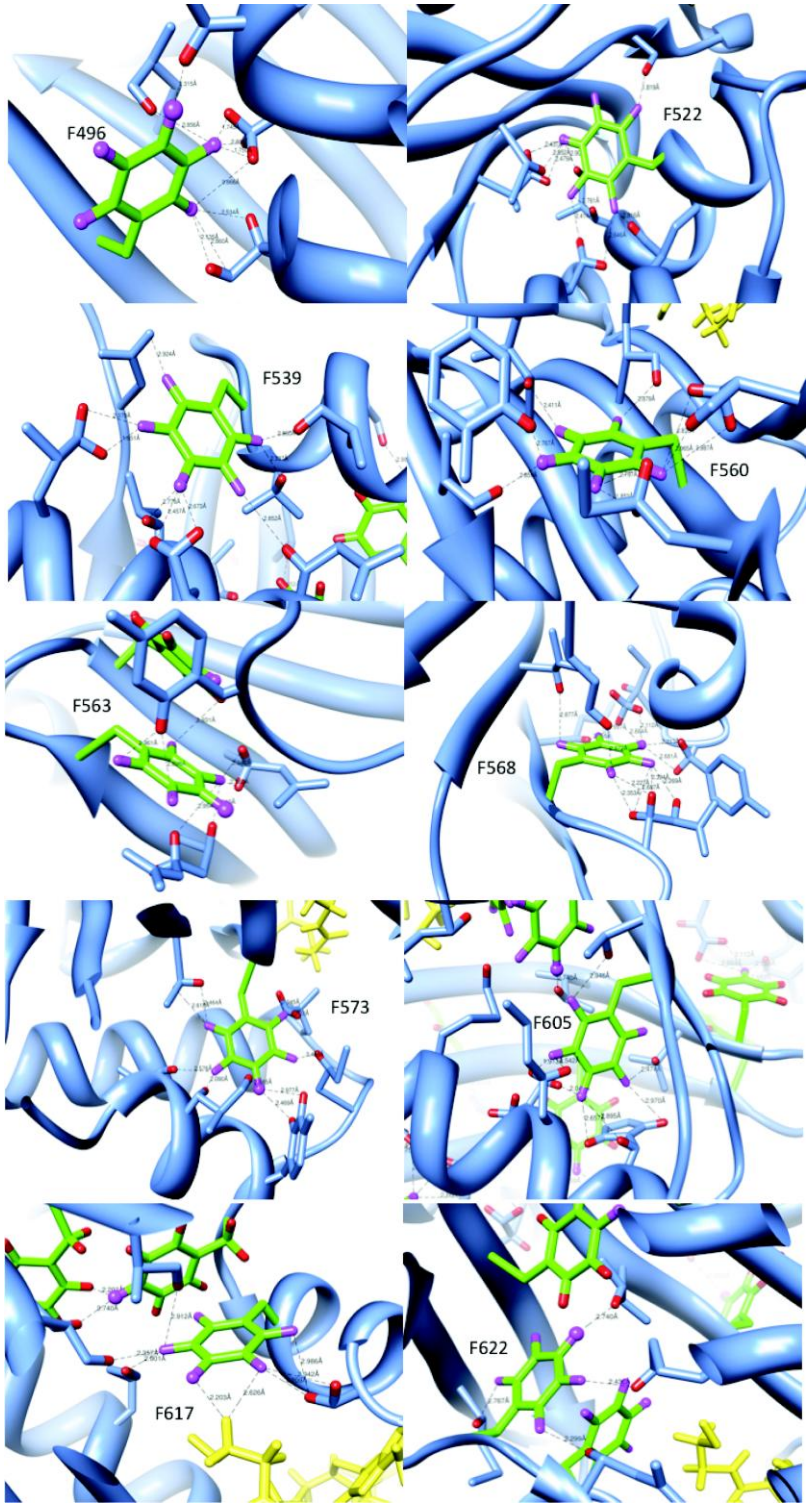
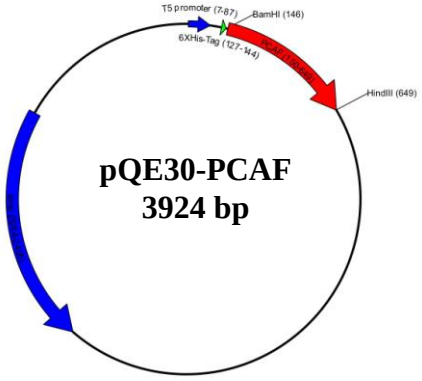


Figure S8. Models of fluorinated phenylalnaines in PCAF using UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.[3, 5] Here we model in pentafluorophenylalanine as a substitute for phenylalanine to assess all ortho-, meta- and para-substituents simultaneously.



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CAAACAAGAAGATCCTGATGTGGCTGGTTGGCCTACA
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PCAF Protein Sequence:
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D P K H K T L A L I K D G R V I G G I C F R M F P S Q G F T E I V F C A V T S N E Q V K G Y G T H L M N H L K E Y H I K
H D I L N F L T Y A D E Y A I G Y F K K Q G F S K E I K I P K T K Y V G Y I K D Y E G A T L M G C E L N P R I P Y T E

Figure S9. Plasmid map and sequence of pQE30-PCAF and translated protein sequence. PCAF gene is highlighted in blue and restriction sites highlighted in red.

Table S1: Purification Yield of PCAF.

Protein	Yield (mg/L)
wt-PCAF	10.4
oFF-PCAF	1.6
mFF-PCAF	2.85
pFF-PCAF	2.6

Table S2a: Unnatural amino acid (UAA) incorporation percentage.

Protein	Incorporation via Amino acid Analysis (%)	Incorporation via Mass Spectrometry (%)
oFF-PCAF	73.3	83.3
mFF-PCAF	ND[↑]	88.0
pFF-PCAF	81.4	90.9

[↑]Amino acid analysis for mFF-PCAF was not determined due to overlap of ammonia peak. Incorporation percent for mFF-PCAF was determined via Mass spectrometry analysis average of three peak heights.

Table S2b: Nanomolar amino acid ratios for oFF percent incorporation (indirect calculation).

	Calculated Value*	Expected Value†	% Incorporation (calculated value/ expected value)	Average oFF incorporation (%)
ratio nmol Phe:Asx	0.1835	0.7692	76.14	73.3
ratio nmol Phe:Val	0.2498	0.9091	72.52	
ratio nmol Phe:Leu	0.2047	0.7143	71.34	

*Calculations are based on presence of Phe peak from AAA chromatogram of oFF-PCAF.
†Calculations are based on presence of theoretical Phe residues in the protein sequence of wt-PCAF.

Table S2c: Nanomolar amino acid ratios for pFF percent incorporation.

pFF nmol[+]	Phe nmol[+]	% incorporation (pFF nmol/[pFF nmol+Phe nmol])
0.319	0.073	81.4

[+] Calculation based on AAA chromatogram of pFF-PCAF

Table S2d: Mass spectrometry peak heights for mFF percent incorporation.

Protein	wt mass peak	UAA mass peak	UAA incorporation (%)	Average UAA incorporation (%)
mFF-PCAF	2915	16654	85	88
	268	3761	93	
	269	1779	86	

Table S3: van der Waals interactions within 3.5 Å

Residue		DISTANCE (Å)
F 617	I 571	3.3
	A 609	3.5
	A 613	1.9
	Y 616	3.2
F 169	L 126	2.6
	K 314	1.8
	M 142	2.9
	Q 172	3.4
	F 174	3.2
	A 165	2.7
	I 166	3.4

Table S4: van der Waals interactions for each phenylalanine residue within 3Å

# H-bonds	ortho-H #, (distance Å)	ortho-F #, (distance Å)	meta-H #, (distance Å)	meta-F #, (distance Å)	para-H #, (distance Å)	para-F #, (distance Å)
F496	3 (2.211, 2.657, 2.885)	**4** (2.135, 2.650, 2.534, 2.866)	4 (1.859, 2.979, 1.859, 2.805)	4 (2.856, 1.745, 2.881, 1.745)	1 (2.448)	1 (2.315)
F522	2(2.153, 2.581)	**4**(1.819,2.816,2.646, 2.393)	4(2.509, 2.625, 2.776, 2.597)	4(2.761,2.414,2.454,2.478)	4(2.593, 2.943, 2.685, 2.908)	4(2.437, 2.900, 2.952,2.479)
F539	2(2.906, 2.553)	2(2.337, 2.880)	2(2.060, 2.640)	**3**(2.852,1.851.2.370)	3(2.900, 2.733, 2.810)	3(2.673,.2457,2.776)
F560	2 (2.333, 2.935)	**5**(2.879, 2.897, 2.965, 2.875,2.252)	3(2.934, 1.993, 2.655)	3(2.411, 2.797, 1.853)	1(2.863)	**2**(2.767, 2.859)
F563	1(2.999)	1(2.961)	5(2.942, 2.854, 2.634, 2.807, 2.550)	5(2.898, 2.954, 2.510,2.562, 2.347)	0	0
F568	3(2.968, 2.361, 2.380)	**4**(2.877, 2.972, 2.227,2.353)	6(2.502, 2.677, 2.469, 2.570, 2.202, 2.618)	6(2.269, 2.687, 2.394, 2.366, 1.997, 2.433)	4(2.445, 2.893, 2.241, 2.952)	4(2.213, 2.681,2.112, 2.884)
F573	2(2.412, 2.865)	**3**(2.233, 2.945, 2.618)	4(2.843, 2.608, 2.288, 2.741)	4(2.644, 2.456, 2.090, 2.576)	1 (2.675)	**3**(2.469, 2.977, 2.985)
F605	0	**1**(2.946)	3(2.614, 2.146, 2.576)	**4**(2.970, 2.474, ,2.542, 1.913)	1(2.878)	**3**(2.042, 2.657, 2.895)
*F617**	*2(2.521, 2.961)*	*3(2.986, 2.411, 2.982)*	*4(2.597,2.355,2.592, 2.986)*	*7(2.912, 2.901, 2.357, 2.942, 2.209,2.993, 2.626)*	*1 (2.218)*	*1(2.203)*
F622	2(2.960, 2.463)	2 (2.767, 2.299)	2(2.551, 2.271)	2(2.173, 2.436)	2(2.950, 2.593)	2(2.740, 2.545)

* interacts with AcCoA and not considered when investigating the extra van der Waals interactions calculations.

References:

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