PCA技术第3应用领域：p53和SHP2 PCA ELISA，用于基础研究和小分子药物发现

蛋白质构象阵列技术在基础研究中的机会

Diseases	Protein involved
Alzheimer‘s disease	Amyloid-β
Parkinson disease	α-Synuclein
Diabetes type 2	Amylin
Amyotrophic lateral sclerosis	Superoxide dismutase
Haemodialysis-related amyloidosis	β2-microglobulin
Cystic fibrosis	Cystic fibrosis transmembrane regulator
Sickle cell anemia	Hemoglobin
Hungtington disease	Huntingtin
Creutzfeldt-Jakob disease	Prion protein
Amyloidosis	Ten other proteins

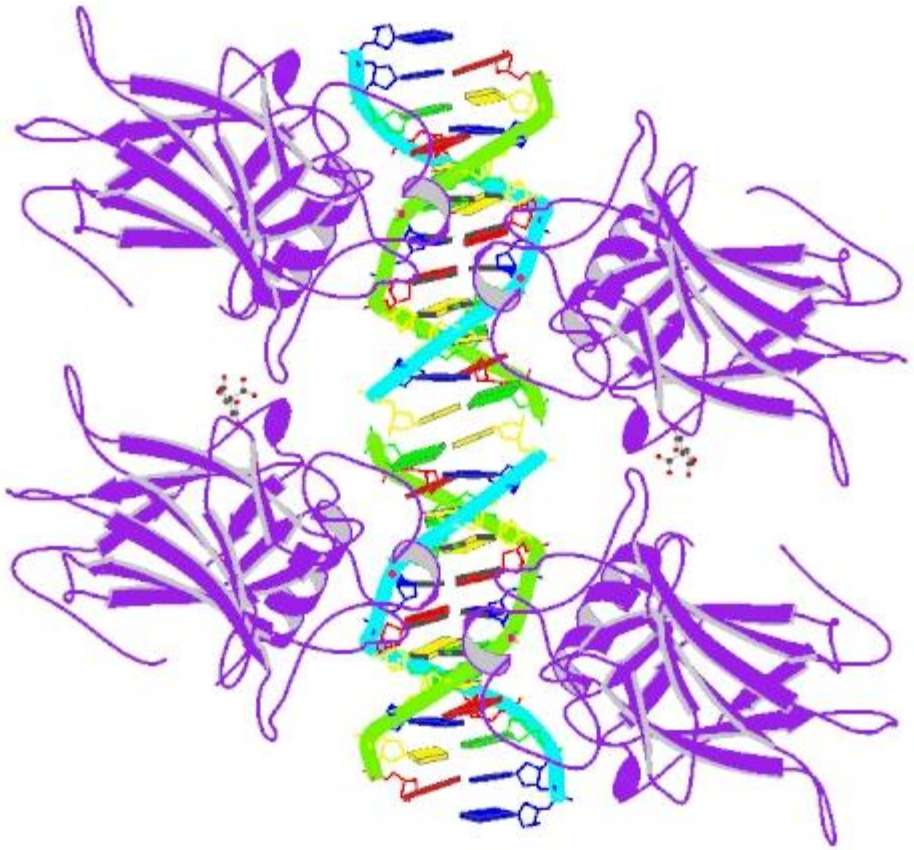
已经确定了50多种与蛋白质构象相关的疾病改变，PCA构象阵列可用于研究疾病机制。

使用一组新的 p53 单克隆抗体进行构象研究 --- From: http://p53.free.fr/our_work/structure.html

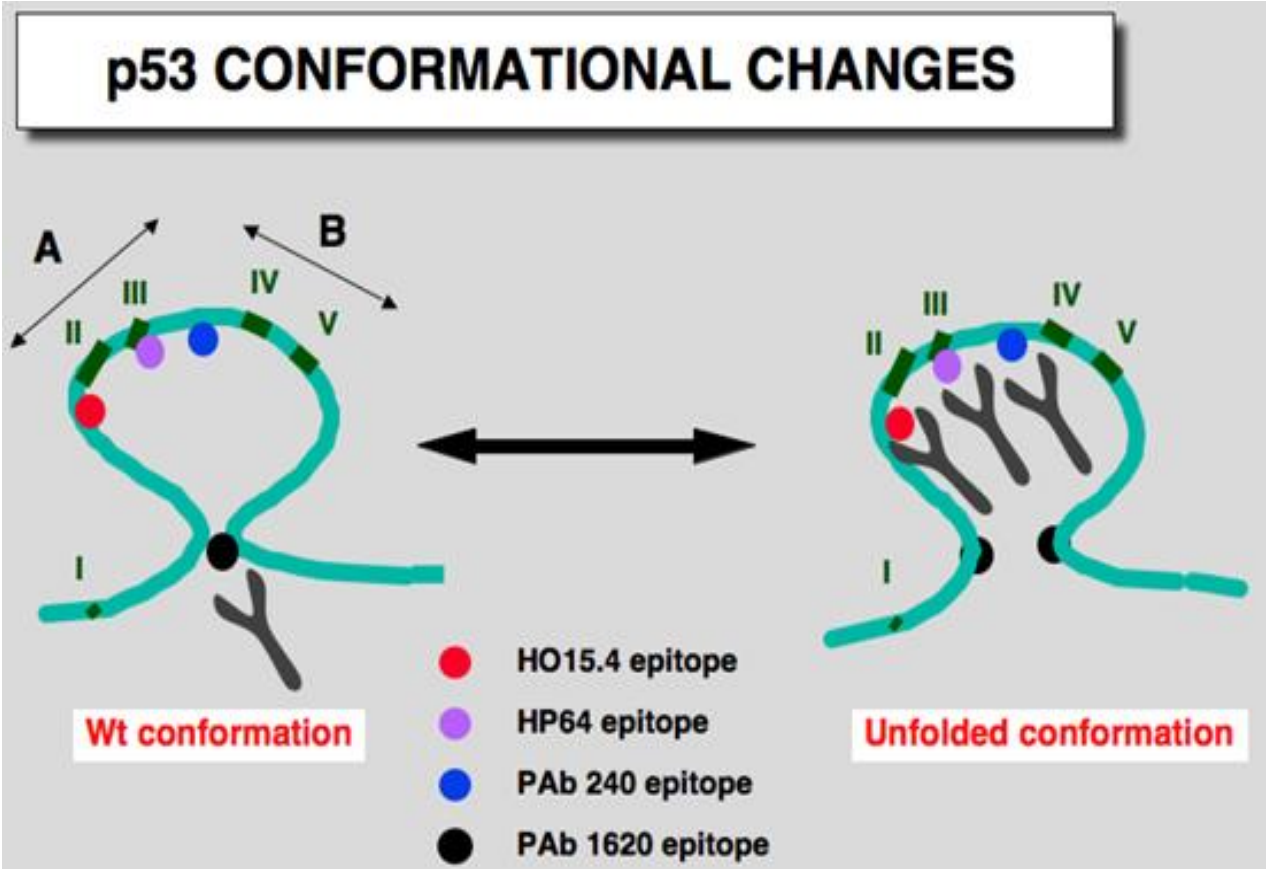
Using a set of overlapping peptides of the human p53 protein, we analysed the epitopes recognized by 18 monoclonal antibodies specific for human p53 , that were produced in our laboratory. We showed that most of these epitopes corresponded to linear antigenic determinants which lie predominantly in the amino- or carboxy-terminus of the p53 protein.

Using a truncated p53 (residues 66 to 361), we selected eight new monoclonal antibodies directed to the central part of the protein. We then identified the epitopes recognized by seven out of these eight antibodies with a set of overlapping peptides. One of these antibodies had an epitope similar to PAb240, whereas the others recognized novel and diverse antigenic determinants. Using a series of 19 p53 mutants, we showed that the behavior of several of the new monoclonal antibodies was similar to that of PAb240 despite their various epitope localizations. This suggests that different mutations in the p53 protein induce an overall conformational change that can be detected by various monoclonal antibodies directed toward the central part of the protein.

P53 与 DNA 结合的三维结构

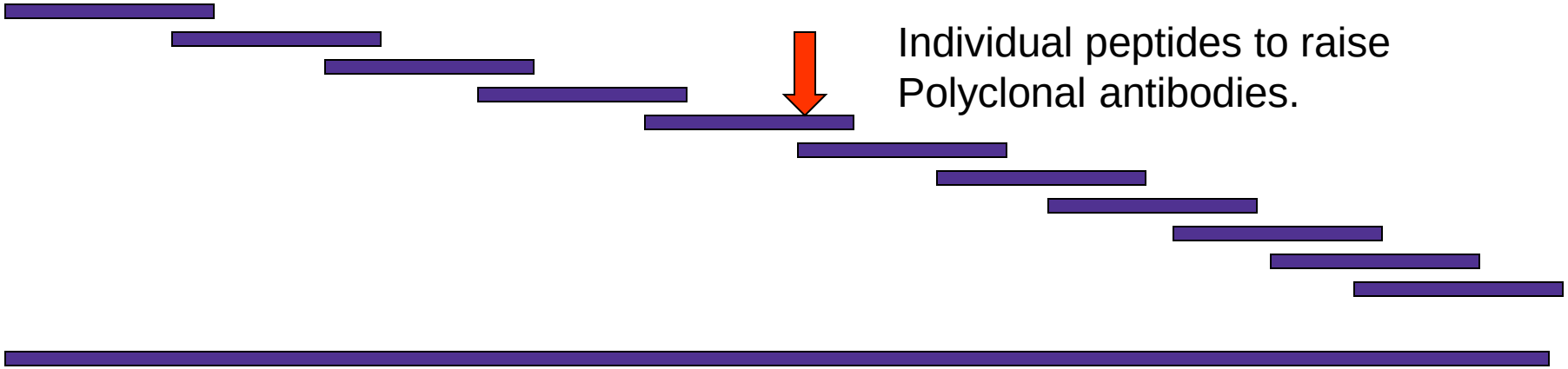


以p53为示例的蛋白质构象阵列技术的学术研究机会



A model for p53 confomation: The amino and carboxy-termini of th p53 protein are highly acessible and are displayed at the surfaceof the prolein. They contain the immunodominant regjons. The central region is ralher compact and allows the formation of he epitope for PAb1620, but epitopes of PAb240, HO15.4, HO3.5, HO13.1 and HP64 are masked. The presence of a specific mutation or the partial denaturation of the p53 protein destroyed the PAb1620 epitope, unmasked those localized in the central region of the protein.
I to V corresponds ro the highly conserved domains of the p53 protein (Soussi et al, 1990). This model is based on the conformational nodel proposd by Milner (1991)

PCA产品的技术开发

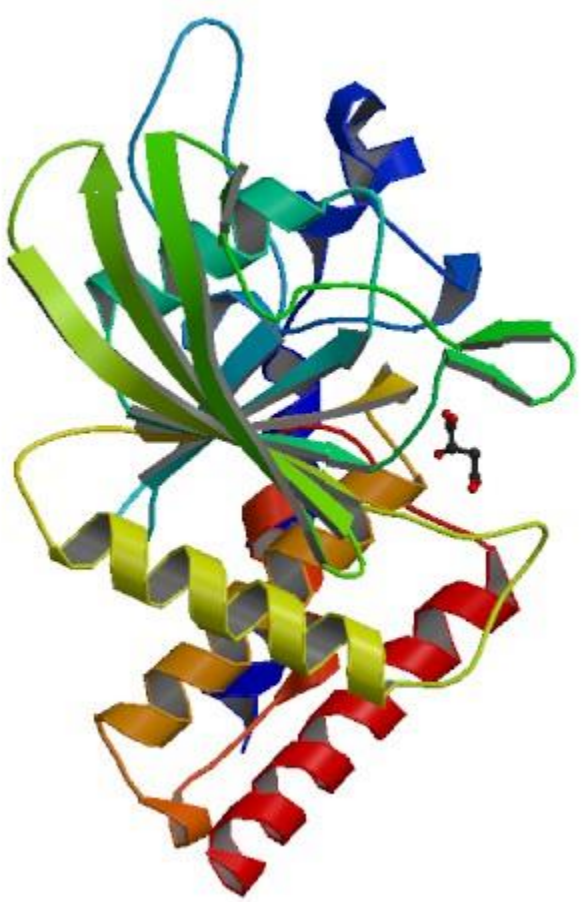


抗体氨基酸序列用于设计具有重叠区域的抗体阵列以覆盖整个目标分子。

TP53 PCA ELISA：抗体特异性检测

	TPPeptide4	TPPeptide 5	TPPeptide7	TPPeptide8	TPPeptide9	TPPeptide10	TPPeptide11	TPPeptide12	TPPeptide13	TPPeptide14	TPPeptide15	TPPeptide16	TPPeptide17
TPserum1	0.541	0.385	0.525	0.168	0.185	0.085	0.132	0.271	0.092	0.854	0.144	0.126	0.11
TPserum2	1.866	1.712	1.315	1.139	1.288	0.34	0.484	0.4	0.36	0.95	0.457	0.309	0.288
TPserum3	1.928	1.701	1.102	1.303	1.234	0.348	0.487	0.502	0.249	0.914	0.369	0.32	0.223
TPserum4	**2.009**	1.474	0.775	0.89	0.767	0.222	0.318	0.243	0.115	0.813	0.203	0.137	0.119
TPserum6	0.315	0.234	0.124	0.13	0.166	0.077	0.222	0.146	0.085	0.853	0.101	0.093	0.123
TPserum7	1.486	0.582	**2.059**	1.843	1.038	0.47	0.84	0.512	0.326	1.026	0.42	0.316	0.469
TPserum8	0.238	0.107	1.012	**2.047**	1.702	0.892	0.88	0.557	0.205	0.949	0.247	0.186	0.195
TPserum9	0.662	0.209	0.28	1.298	**2.096**	1.055	0.605	0.367	0.173	0.95	0.385	0.182	0.16
TPserum10	0.94	0.304	0.263	1.228	1.032	**2.137**	2.027	1.277	1.009	0.953	0.636	0.205	0.13
TPserum11	0.458	0.106	0.11	0.176	0.155	1.581	**1.971**	0.368	1.11	0.548	0.132	0.084	0.161
TPserum12	0.353	0.178	0.157	0.313	0.231	0.766	0.802	**2.031**	0.464	0.747	0.129	0.421	0.1
TPserum13	0.406	0.085	0.085	0.09	0.095	0.24	0.19	0.404	**2.101**	1.851	1.252	0.35	0.117
TPserum14	0.298	0.156	0.143	0.224	0.178	0.322	0.562	0.54	0.972	**2.128**	2.032	1.482	0.967
TPserum15	0.268	0.161	0.13	0.201	0.165	0.154	0.218	0.258	0.142	1.519	**2.166**	1.659	0.691
TPserum16	0.165	0.09	0.085	0.115	0.099	0.078	0.144	0.129	0.068	0.915	1.146	**0.371**	2.073
TPserum17	1.955	0.115	0.358	0.119	0.131	0.097	0.117	0.173	0.091	0.811	0.757	2.152	**2.054**

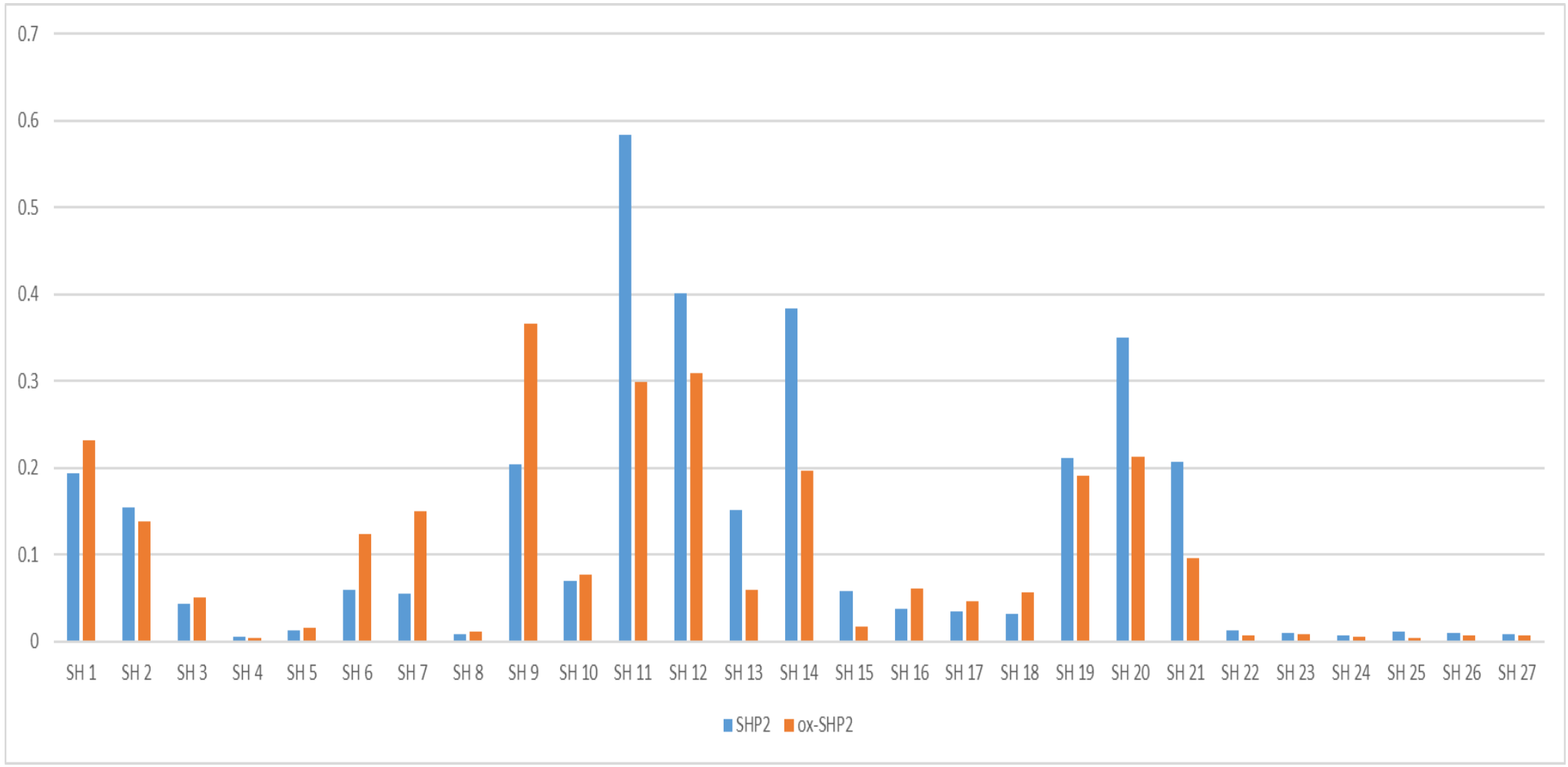
SHP2 晶体结构式



SHP2 PCA ELISA：抗体特异性检测

	PEP1	PEP2	PEP3	PEP4	PEP5	PEP6	PEP7	PEP8	PEP9	PEP10	PEP11	PEP12	PEP13	PEP14	PEP15	PEP16	PEP17	PEP18	PEP19	PEP20	PEP21	PEP22	PEP23	PEP24
SHSer1	**2.025**	0.643	0.54	0.257	0.197	0.167	0.148	0.248	0.109	0.139	0.177	0.084	0.823	1.168	0.441	0.464	0.155	0.211	0.252	0.197	0.118	0.159	0.221	1.691
SHSer2	1.081	**2.067**	1.73	0.922	0.56	0.505	1.397	0.31	0.202	0.168	0.153	0.226	0.141	1.182	0.615	0.516	0.184	0.174	0.255	0.205	0.152	0.138	0.217	1.472
SHSer3	0.552	1.275	**1.92**	1.104	0.555	0.239	0.301	0.412	0.444	0.356	0.105	0.121	0.149	1.143	0.608	0.464	0.197	0.125	0.184	0.206	0.111	0.125	0.31	1.481
SHSer4	0.401	0.357	0.89	**1.821**	0.975	0.271	0.185	0.269	0.199	0.074	0.095	0.103	0.626	1.065	0.505	0.4	0.151	0.087	0.121	0.132	0.094	0.099	0.192	1.376
SHSer5	0.391	0.387	1.15	0.869	**1.531**	1.036	0.553	0.388	0.185	0.219	0.123	0.151	0.232	1.017	0.695	0.459	0.242	0.136	0.167	0.227	0.148	0.129	0.363	1.448
SHSer6	0.408	0.292	0.59	0.256	0.487	**2.06**	1.969	1.136	0.493	0.41	0.286	0.268	0.326	1.157	0.652	0.529	0.185	0.15	0.177	0.208	0.203	0.135	0.238	1.545
SHSer7	0.504	0.364	0.49	0.158	0.123	0.741	**1.317**	1.247	0.5	0.32	0.212	0.236	0.79	1.379	0.799	0.541	0.177	0.137	0.159	0.147	0.139	0.126	0.228	1.662
SHSer8	0.477	0.385	0.56	0.23	0.19	0.658	1.077	**1.965**	1.753	1.325	1.186	1.391	1.278	1.48	1.004	0.648	0.383	0.377	0.485	0.407	0.471	0.352	0.663	1.624
SHSer9	0.398	0.238	0.4	0.464	0.166	0.401	0.504	0.936	**2.063**	1.69	0.922	0.575	0.868	0.665	0.803	0.564	0.198	0.188	0.312	0.265	0.192	0.204	0.316	1.621
SHSer10	0.42	0.292	0.48	0.279	0.234	0.729	0.675	0.801	1.878	**2.014**	1.295	0.698	0.906	1.747	1.136	0.627	0.222	0.155	0.234	0.239	0.159	0.165	0.392	1.586
SHSer11	0.451	0.25	0.37	0.173	0.12	0.237	0.196	0.36	1.062	0.938	**2.105**	1.507	0.328	0.885	0.721	0.526	0.168	0.128	0.154	0.152	0.121	0.175	0.248	1.573
SHSer12	0.378	0.261	0.43	0.167	0.157	0.209	0.175	0.341	0.822	0.443	1.353	**2.03**	1.389	1.129	1.091	0.818	0.3	0.306	0.924	0.323	0.202	0.213	0.482	1.618
SHSer13	0.438	0.262	0.42	0.168	0.124	0.324	0.218	0.365	0.582	0.288	0.954	1.569	**1.813**	0.951	0.882	0.652	0.207	0.18	0.426	0.209	0.135	0.163	0.356	1.558
SHSer14	0.452	0.258	0.4	0.175	0.146	0.203	0.17	0.342	0.445	0.297	0.685	1.384	0.722	**2.021**	1.848	1.361	0.435	0.304	0.346	0.869	0.218	0.229	0.318	1.533
SHSer15	0.463	0.255	0.45	0.149	0.121	0.283	0.246	0.361	0.529	0.27	0.376	0.88	0.567	1.669	**1.975**	1.529	0.614	0.468	0.642	0.326	0.204	0.226	0.296	1.581
SHSer16	0.376	0.247	0.39	0.171	0.146	0.307	0.285	0.362	0.556	0.29	0.441	0.772	0.515	1.286	1.402	**1.845**	0.908	0.637	0.722	0.309	0.212	0.266	0.325	1.645
SHSer17	0.41	0.276	0.44	0.213	0.188	0.255	0.263	0.314	0.298	0.193	0.372	0.334	0.923	0.892	0.961	0.787	**2.063**	1.271	0.664	0.354	0.216	0.446	0.323	1.604
SHSer18	0.495	0.271	0.43	0.172	0.152	0.213	0.175	0.259	0.241	0.117	0.218	0.202	0.957	0.94	0.937	0.617	1.124	**1.943**	1.393	0.476	0.213	0.234	0.3	1.677
SHSer19	0.476	0.285	0.45	0.167	0.147	0.218	0.164	0.284	0.297	0.134	0.236	0.278	1.282	1.057	0.998	0.667	0.952	0.887	**1.446**	0.478	0.171	0.182	0.295	1.589
SHSer20	0.44	0.277	0.41	0.213	0.173	0.231	0.175	0.287	0.283	0.153	0.259	0.284	0.85	1.15	0.889	0.585	0.808	0.565	0.661	**2.127**	1.948	1.485	0.715	1.644
SHSer21	0.47	0.246	0.57	0.223	0.16	0.184	0.147	0.283	0.238	0.153	0.254	0.236	0.78	0.908	1.209	0.508	0.391	0.329	0.388	1.631	**2.089**	1.752	0.754	1.538
SHSer22	0.499	0.298	0.44	0.219	0.119	0.146	0.098	0.268	0.17	0.106	0.202	0.175	0.95	1.038	1.001	0.547	0.283	0.215	0.251	0.909	1.881	**1.642**	0.486	1.602
SHSer23	0.494	0.31	0.43	0.212	0.134	0.14	0.096	0.241	0.128	0.108	0.197	0.111	1.149	1.144	0.93	0.524	0.178	0.134	0.157	0.362	0.968	0.68	**2.081**	2.065
SHSer24	0.451	0.317	0.48	0.226	0.101	0.112	0.073	0.253	0.123	0.089	0.207	0.1	1.22	1.174	0.952	0.577	0.192	0.131	0.15	0.297	0.605	0.464	1.441	**2.059**

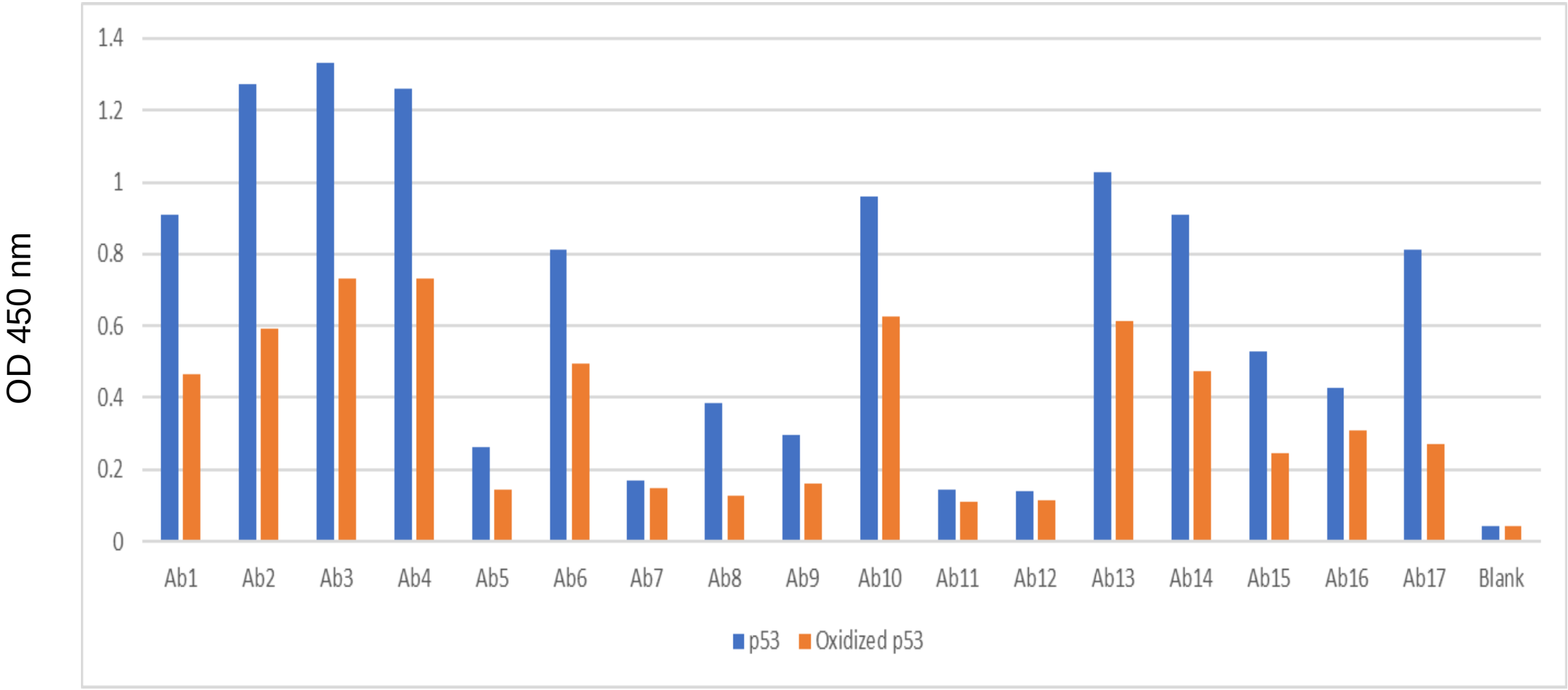
SHP2 HOS 的 PCA 酶联免疫吸附测定



OD 450 nm

Antibody Number

使用p53 PCA酶联免疫吸附测定法进行 p53 HOS分析



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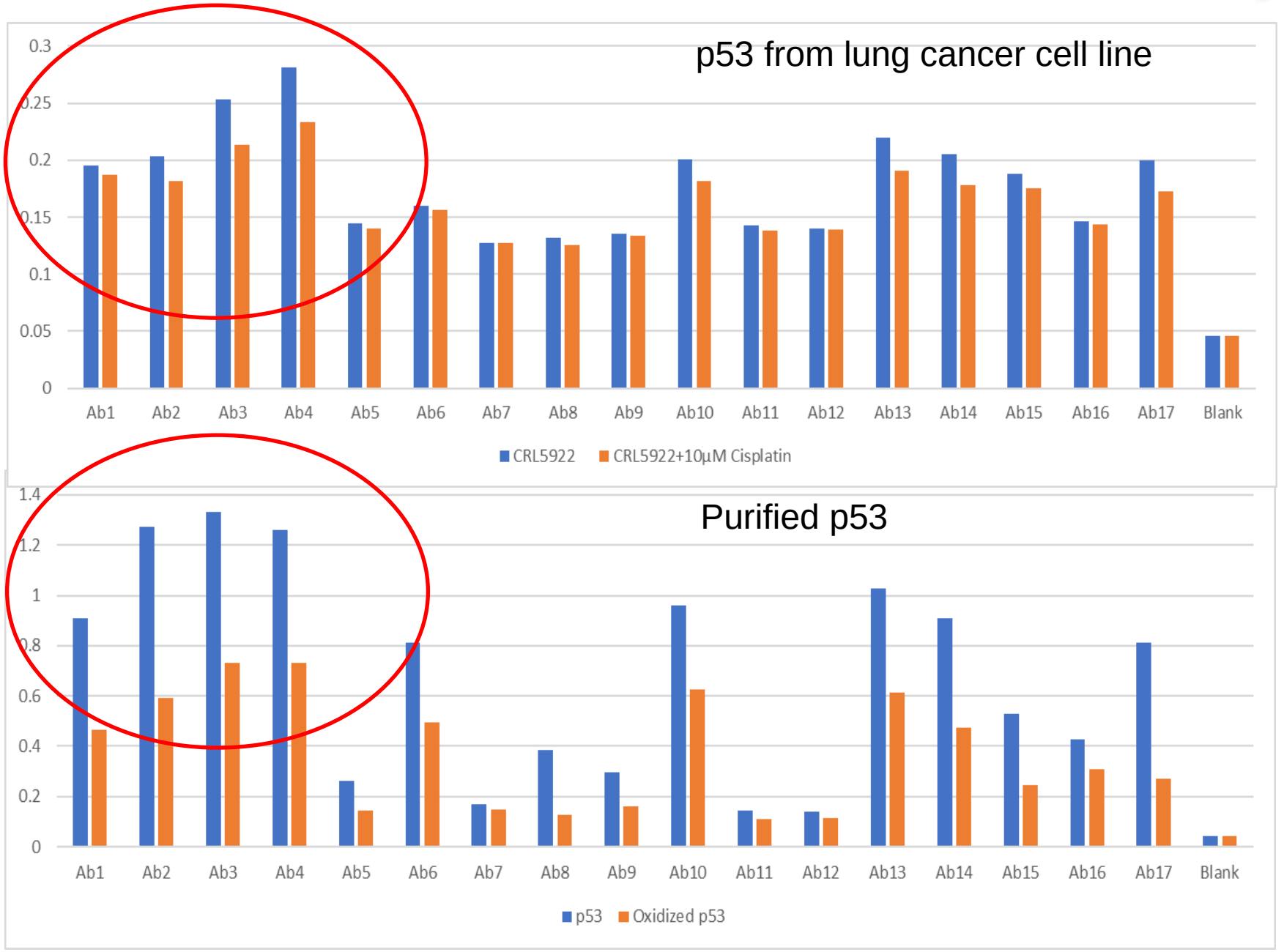

结论

- P53 和 SHP2 PCA酶联免疫吸附测定法可以系统的研究这两个重要肿瘤靶点的三维结构变化。
- 这两个方法可以用来快速筛选通过破坏蛋白质三维构像抑制肿瘤的小分子药物
- PCA 技术平台可以快速对任何重要的蛋白质进行产品构建，成为基础研究和小分子药物筛选的重要工具