Profiling epitope-specific antibody responses against malaria utilizing high-density peptide arrays

Kirsten Heiss[1*], Thomas Jaenisch[2*], Nico Fischer[2], Carolin Geiger[2], Gregor Jainta[1], Leszek Rychlewski[3], Ali Sie´[4], Boubacar Coulibaly[4], Lucjan S. Wyrwicz[5], Frank Breitling[6], and Felix F. Loeffler[7]

[1]PEPperPRINT GmbH, Heidelberg, Germany; [2]Center for Infectious Diseases & Heidelberg Institute of Global Health, Heidelberg University Hospital, Germany; [3]BioInfoBank Institute, Poznań, Poland; [4]Centre de Recherche en Santé de Nouna, Nouna, Burkina Faso; [5]Department of Oncology and Radiotherapy, M Sklodowska Curie Memorial Cancer Center, Warsaw, Poland; [6]Institute of Microstructure Technology, Karlsruhe Institute of Technology, Karlsruhe, Germany; [7]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Abstract

Antibody-mediated defense mechanisms play an important role in combating malaria infection attacking the parasite at multiple life cycle stages. By reducing parasitemia and clinical symptoms, humoral responses are the key immune effector mechanisms at the pathogenic asexual blood stage. Intensive research focusses on the identification of target antigens of protective anti-plasmodial immune responses, but the entire picture is still incomplete.

High-density peptide microarrays represent an emerging tool, which can be applied to screen a large diversity of linear and conformational antibody epitopes. The technology provides a multifaceted application spectrum, where antibody reactivities to tens of thousands of peptides can be monitored simultaneously in a single assay. We used the peptide microarray technology to better characterize epitopes associated with naturally acquired immunity (NAI). We analyzed the peptide array profiles of serum samples from individuals living in a malaria-endemic area and compared the responses to malaria-naïve individuals. The results show distinct antibody patterns according to immune status for peptides predicted by applying different algorithms, as well as for peptides derived from well-known vaccine candidates. We demonstrated that peptide arrays can be applied as a novel screening method for the identification of new immunogenic antibody epitopes. The discovery of the antigens and/or antigenic motifs that are responsible for protective immune responses will ultimately create new opportunities for vaccine development.

Study design

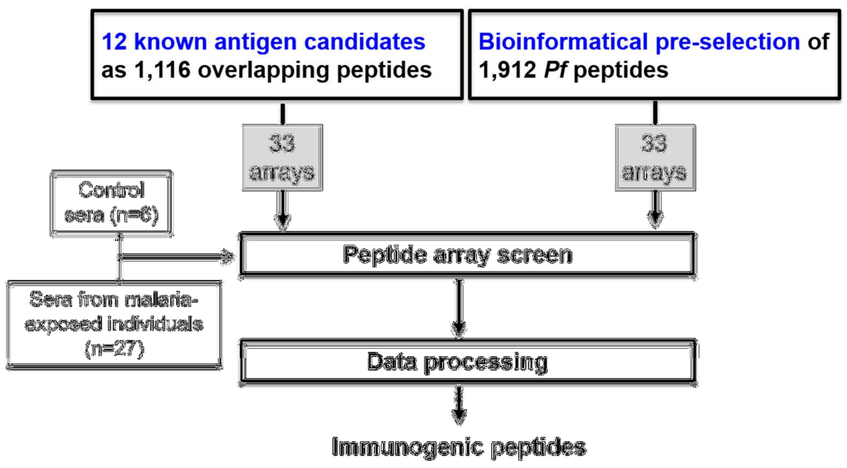
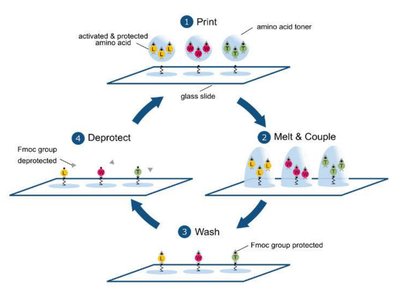


Figure 1. Applied approaches to profile antibody responses in naturally acquired immunity. We selected 12 *P. falciparum* proteins (3D7 strain), which are known vaccine candidates, and mapped them as overlapping peptides on arrays. Pre-selected peptides derived from the *P. falciparum* proteome were also printed on arrays. Peptide microarrays were probed with sera from Malaria-naïve, European individuals (Control) and Malaria-exposed individuals from Nouna, Burkina Faso. Significantly recognized epitopes were identified applying an asinh transformation followed by a two-sided Welch's t-test.

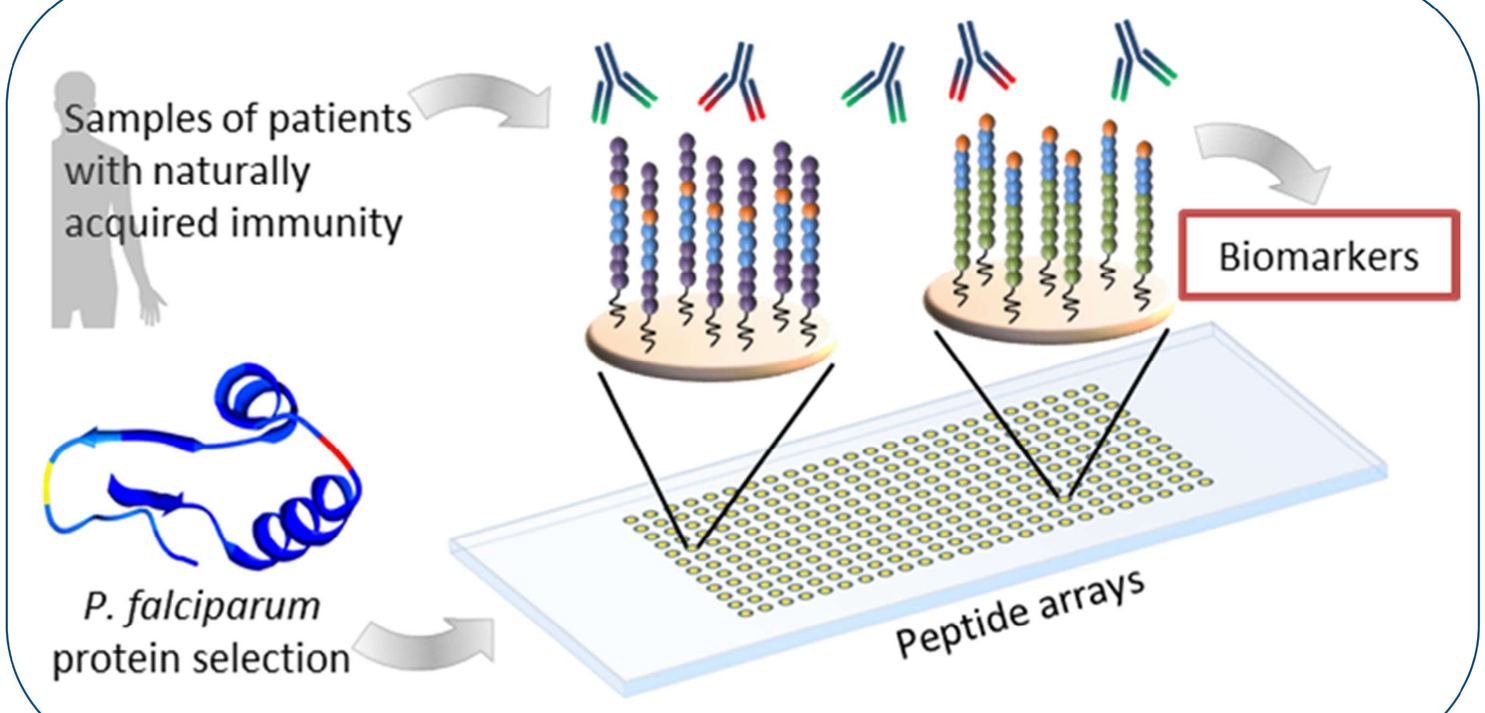
Peptide microarray technology



Benefits

- very low material consumption
- high spot density / high content (1,200 peptides/cm^2)
- digital printing flexibility, fast production times
- high peptide quality with routine double couplings

Figure 2. High-density PEPperCHIP® peptide microarrays are generated by digital laser printing on standard glass slides using a proprietary laser printer comprising 24 cartridges filled with individual amino acid toners. For array production, the amino acid toners are simultaneously printed with high precision on their respective positions on the glass slides (1). Peptide synthesis is initiated by melting the toner particles at 90°C (2) releasing amino acids which are then available for coupling to the previous amino acid. Subsequent washing steps remove excess building blocks (3). Finally, the protection group is removed (4).



Discovery of linear epitopes significantly recognized by antibodies from disease-protected individuals

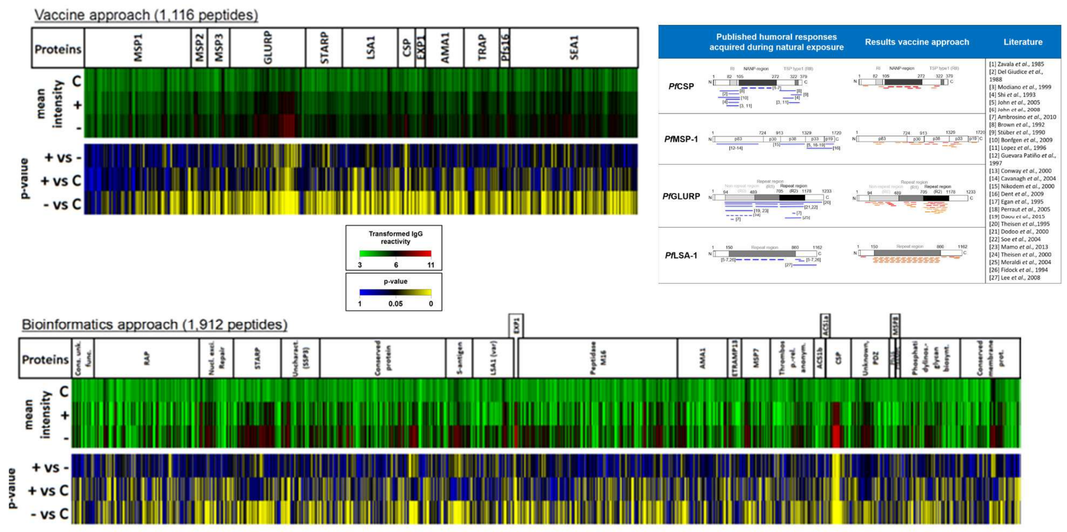
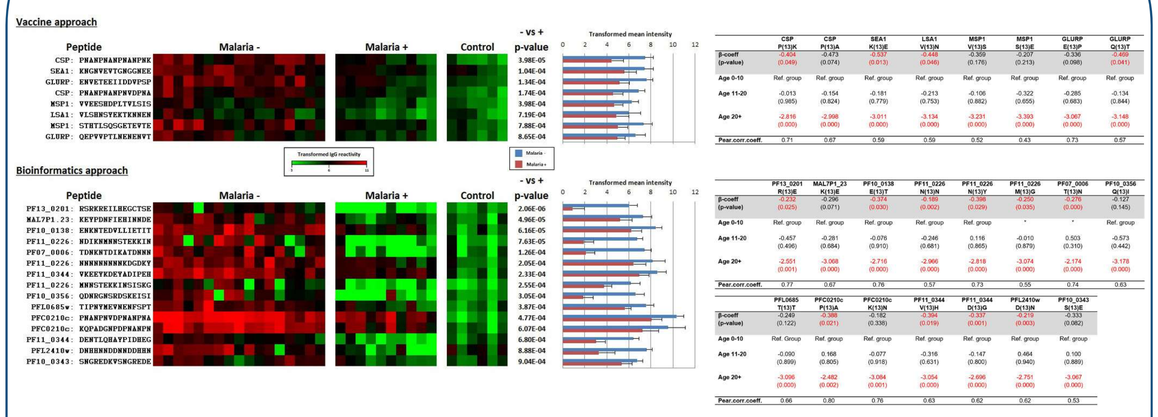


Figure 3. Heat-maps show the mean fluorescence intensity for each individual peptide detected for the respective group. C; Control (Malaria-naïve, n=6). +; Individuals with high parasitemia assuming low immunity (>2000 P/µl; n=10). -; Individuals with low parasitemia assuming NAI (<200 P/µl; n=17). For the vaccine approach, a comparison of the results with the literature for selected antigens is shown. Described immunogenic sequence stretches are shown in blue. Identified epitopes from the vaccine approach are shown in orange (p-value <0.05) and red (p-value <0.01).

Identified top hit peptides and age adjustment



	CSP P(13)K	CSP P(13)A	SEA1 K(13)E	LSA1 V(13)N	MSP1 V(13)S	MSP1 S(13)E	GLURP E(13)P	GLURP Q(13)T
β-coeff (p-value)	-0.404 (0.049)	-0.473 (0.074)	-0.837 (0.013)	-0.448 (0.046)	-0.309 (0.178)	-0.207 (0.213)	-0.336 (0.098)	-0.469 (0.041)
Age 0-10	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group
Age 11-20	-0.013 (0.985)	-0.154 (0.824)	-0.181 (0.779)	-0.213 (0.753)	-0.105 (0.882)	-0.322 (0.655)	-0.285 (0.683)	-0.134 (0.844)
Age 20+	-2.816 (0.003)	-2.998 (0.000)	-3.011 (0.000)	-3.134 (0.000)	-3.231 (0.000)	-3.393 (0.000)	-3.067 (0.000)	-3.148 (0.000)
Pear.corr.coeff.	0.71	0.67	0.59	0.59	0.52	0.43	0.73	0.57

	PF13_0201 R(13)E	MAL7P1_23 K(13)E	PF10_0138 E(13)T	PF11_0226 N(13)N	PF11_0226 N(13)Y	PF11_0226 M(13)G	PF07_0006 T(13)N	PF10_0356 Q(13)I
β-coeff (p-value)	-0.232 (0.329)	-0.296 (0.071)	-0.374 (0.092)	-0.189 (0.002)	-0.398 (0.029)	-0.250 (0.035)	-0.276 (0.009)	-0.127 (0.145)
Age 0-10	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group	*	*	Ref. group
Age 11-20	-0.457 (0.496)	-0.281 (0.084)	-0.076 (0.910)	-0.246 (0.681)	0.116 (0.865)	-0.010 (0.879)	0.503 (0.310)	-0.573 (0.442)
Age 20+	-2.551 (0.001)	-3.068 (0.000)	-2.716 (0.000)	-2.966 (0.000)	-2.818 (0.000)	-3.074 (0.000)	-2.174 (0.000)	-3.178 (0.000)
Pear.corr.coeff.	0.77	0.67	0.76	0.57	0.73	0.55	0.74	0.63

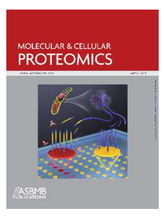
	PFL0685 T(13)T	PFC0210c P(13)A	PFC0210c K(13)N	PF11_0344 V(13)H	PF11_0344 D(13)G	PFL2410w D(13)N	PF10_0343 S(13)E
β-coeff (p-value)	-0.249 (0.122)	-0.388 (0.021)	-0.182 (0.338)	-0.344 (0.019)	-0.337 (0.001)	-0.218 (0.003)	-0.333 (0.082)
Age 0-10	Ref. Group	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group	Ref. group
Age 11-20	-0.090 (0.899)	0.168 (0.805)	-0.077 (0.918)	-0.316 (0.631)	-0.147 (0.800)	0.464 (0.940)	0.100 (0.889)
Age 20+	-3.096 (0.005)	-2.482 (0.002)	-3.084 (0.001)	-3.054 (0.000)	-2.696 (0.000)	-2.751 (0.000)	-3.067 (0.000)
Pear.corr.coeff.	0.66	0.80	0.76	0.63	0.62	0.62	0.53

Figure 4. Individual antibody responses against the top p-value peptides comparing malaria positive and malaria negative samples from respective approaches. The vaccine candidate approach rendered 8 peptides with a p-value <10^{-3}, the bioinformatics approach 15 peptides with p-value <10^{-3}. Individual transformed IgG reactivities are shown in a heat map (for Malaria negative, Malaria positive and Control). The mean transformed reactivities are shown as bar charts (blue: mean transformed reacitivies Malaria negative; red: mean transformed reactivities Malaria positive).

The **tables** show the age adjustment for the respective top hit peptides. The association between parasite density (log10 transformed) as the dependent variable and the peptide and age as explanatory variables was analyzed by applying a multivariable regression analysis. Age was stratified into three groups (0-10, 11-20 and 20+ years) based on sample size and taking into account the development of NAI against malaria with age. The majority of the top hit peptides still reach statistical significance after age adjustment (red highlights p-value < 0.05).

Summary & Conclusion

- First study using high-density peptide arrays to profile anti-plasmodial antibody responses in NAI against linear epitopes
- Identification of novel, highly immunogenic epitopes in both well-known vaccine candidates and previously uncharacterized antigens
- The peptide-based approach allows to unravel differential antibody binding to specific epitopes which might be missed when using the entire antigen



Jaenisch T*., Heiss K*. *et al*., 2019