Development of a Novel In Silico Docking Simulation Model for the Fine HIV-1 Cytotoxic T Lymphocyte Epitope Mapping

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Abstract

Introduction: Class I HLA’s polymorphism has hampered CTL epitope mapping with laborious experiments. Objectives are 1) to evaluate the novel in silico model in predicting previously reported epitopes in comparison with existing program, and 2) to apply the model to predict optimal epitopes with HLA using experimental results.

Materials and Methods: We have developed a novel in silico epitope prediction method, based on HLA crystal structure and a peptide docking simulation model, calculating the peptide-HLA binding affinity at four amino acid residues in each terminal. It was applied to predict 52 HIV best-defined CTL epitopes from 15-mer overlapping peptides, and its predictive ability was compared with the HLA binding motif-based program of HLArestrictor. It was then used to predict HIV-1 Gag optimal epitopes from previous ELISpot results.

Results: 43/52 (82.7%) epitopes were detected by the novel model, whereas 37 (71.2%) by HLArestrictor. We also found a significant reduction in epitope detection rates for longer epitopes in HLArestrictor (p = 0.027), but not in the novel model. Improved epitope prediction was also found by introducing both models, especially in specificity (p<0.001). Eight peptides were predicted as novel, immunodominant epitopes in both models.

Discussion: This novel model can predict optimal CTL epitopes, which were not detected by an existing program. This model is potentially useful not only for narrowing down optimal epitopes, but predicting rare HLA alleles with less information. By introducing different principal models, epitope prediction will be more precise.


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Introduction

Cytotoxic T lymphocytes (CTLs) play a crucial role in HIV replication control by eliminating virus-infected cells by recognizing class I Human Leukocyte Antigen (HLA) molecule-viral peptides (≡ epitope) complex. This response is thought to be a major determinant of the viral set point, and consequent disease progression [1]. However the efficacy of the CTL response is affected by the extent of polymorphisms in HLA loci and viral sequences. The HLA region is found on chromosome 6 and is the most polymorphic loci in the human genome [2]: each individual expresses up to six different class I alleles out of a vast pool of allelic variants, the reported number of which reaches 5,399 for class I HLA molecules (1,757 of HLA-A, 2,338 of HLA-B, and 1,304 of HLA-C alleles) [3]. In addition, the extensive diversity of HIV-1 owing to its extreme capacity to mutate has led to a reported 13 prototype clades and 43 circulating recombinant forms (CRFs) [4]. Despite such HLA polymorphism and HIV viral diversity environment, recent genome wide association study (GWAS) reported the best contribution of class I HLA for viral control, suggesting the importance of CTL epitope mapping with responsible HLA information [5]. Several major HIV-1 epitopes and their restricting HLA alleles have been defined through fine epitope mapping; 1,344 epitopes and their restricting HLA alleles have been reported as of February 2012 (CTL Epitopes. Los Alamos National Lab. http://www.hiv.lanl.gov/). The limitation of the dataset currently available however, is that the majority of these epitope/HLA combinations are derived from subtype B-infected Caucasians or C-infected Africans, and epitope information from other subtypes or ethnicities is rare.

The traditional, in vitro method of epitope detection involves using a matrix of overlapping peptides (OLPs) encoding viral proteins in Enzyme-Linked Immunospot (ELISpot) assays to
identify a single candidate peptide, from which the 8-11mer epitope is mapped down. This is typically followed by the confirmation of the restricting HLA alleles using tetramers or in a $^{51}Cr$ release assay using peptide-specific lines [6,7]. It is a difficult and labor-intensive process, particularly time-consuming in the case of epitopes restricted by rare HLA alleles because of the limited number of samples available.

Recently, alternative, in silico models for epitope prediction have been developed [8]. These can broadly be divided into two models; the first is an algorithm based on the peptide-binding motif, and the second is a structural algorithm model based on the crystal structure of HLA molecules. The former is characterized by the use of motif matrices deduced from refined motifs based on the pool sequence, enlisting optimal amino acid sequences at anchor positions in specific HLA alleles. An example of such an algorithm is the SYFPEITHI [9] database, which predicts the HLA-binding affinities of peptides by ranking them according to the presence of primary and secondary anchor amino acids. However these models are based on reported epitopes and their restricting HLA alleles, so their predictions are powerful in the context of well-published HLA alleles but not suitable against rare or novel alleles with little previous information. Another model of epitope prediction is the binding affinity model, which calculates the peptides' binding affinity and scores it using quantitative matrices (QMs), a well-known example being the NetMHC [10,11] or the HLArestrictor [12]. This model scores binding strength as binding affinity with thresholds to differentiate strong binding peptides and weak ones in each calculation.

On the other hand, the structural algorithm model does not require binding motif information, which is advantageous for the definition of epitopes restricted by HLA alleles with little published epitope information. Recently, a docking simulation model (DSM) which takes into consideration binding energy such as electrostatic interactions and van der Waals (vdw) interactions, together with the crystal structure of HLA alleles, has been developed [13–17].

Our objectives here are 1) to evaluate the novel in silico DSM in predicting previously reported best-defined epitopes in comparison with existing binding motif-based program, and 2) to apply the model to predict optimal size of the epitopes and restricting HLA alleles using results obtained from our previous study in a HIV-1 CRF01_AE-infected Thai cohort.

**Materials and Methods**

**Ethic Statement**

This study was approved by Thai Ministry of Public Health Ethics Committee. Written informed consent was obtained from all patients after explaining the purpose and expected consequences of the study.

**Computational program and calculation**

We used the commercial softwares Molecular Operating Environment® (MOE) (CCG Inc., Montreal, Canada) and MOE-ASEDock® (Ryoka System Inc., Tokyo, Japan) for the molecular binding affinity calculation [18]. HLA's 3D models were obtained from the X-ray crystallography database in MOE's library [1OGA for HLA-A*02:01, 1IQ9 for HLA-A*11:01, 2BCK for HLA-A*24:02, 1XR9 for HLA-B*15:01, 1JGE for HLA-B*27:05, 2CIR for HLA-B*35:01, 1E27 for HLA-B*52:01, 2RX for HLA-B*57:01, and 1EFX for HLA-C*0702]. In cases where the original X-ray crystallography information was unavailable, we generated a 3D structural model using highly homologous HLA alleles as template, using rotamer explorer or homology modeling to reconstruct their structures by changing sequential differences sites, a method originally used in the point mutation program attached in MOE AMBER99 [19] for force field calculations. For solvent effect energy calculation, a generalized Born model [20], were introduced. As an indicator of the affinity between epitope candidate peptides and the class I HLA allele, we measured the U_dock score [U_ele (electric energy)+U_vdw (van der Waals energy)+U_solv (Solvation energy)+U_strain (Strain energy)] (kcal/mol) [18]. We calculated the U_dock score of four residues at each N- and C-terminal, spanning the anchor position at each of the terminals, and scored the sum of them as binding affinity. A lower score indicates a higher affinity between the HLA molecule and peptides.

**Evaluation of the novel DSM through an analysis of best-defined HIV CTL epitopes and their restricting HLA alleles**

For the quality evaluation of this novel program, we first calculated the U_dock score for 52 best-defined HIV epitopes restricted by the alleles HLA-A*02:01, HLA-A*11:01, HLA-A*24:02, HLA-B*15:01, HLA-B*27:05, HLA-B*35:01 and HLA-B*57:01 as enlisted in Los Alamos database (CTL Epitopes, Los Alamos National Lab. http://www.hiv.lanl.gov/). We calculated the U_dock score between the restricting HLA alleles and the 8 to 11-mer peptides within 15-mer peptides of the viral strain HXB2, in which best-defined epitopes were included. 26 variants of 8 to 11-mer peptides were calculated in one HLA and 15-mer peptide combination, then the lowest U_dock score was ranked as the 1st and the highest score as the 26th in each calculation (Figure 1). Combinations that ranked within the top five were regarded as positive. In parallel with our DSM, we also performed epitope prediction using the latest artificial neural network (ANN) model, the HLArestrictor [12], using the affinity thresholds of Strong Binder (SB), Weak Binder (WB), Combined Binder (CB) and Non-binder (NB), according to their definitions.

We evaluated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each best-defined epitope prediction using the DSM, HLArestrictor, as well as those defined as dual positive by both models.

**Analysis of in vitro HIV-1 CRF01_AE Gag epitope candidates by using both in silico epitope prediction models**

We then applied both the DSM and the HLArestrictor to predict the optimal size of epitopes, based on results obtained from our previous study [21], in which 31 candidate epitopes were detected by ELSpot assays using Gag 15-mer OLPs and their HLA associations detected by Fisher’s exact test in a cohort of 137 (107 female and 30 male) HIV-1 CRF01_AE-infected Thais. All were chronically infected and treatment naive, with median 461/ul CD4+T cell count (range 204–1,191) and 4.2 log copies/ml viral load (2.6–5.9).

**Epitope prediction for the immunogenic Gag OLP p24276–285 MYSPVSILDI using a $^{51}Cr$ release assay and both in silico models**

In our previous study [21], the 15-mer peptides Gag p24271–285 NKIVRMYSPVSILDI (NI15) and p24 276–290 MYSPVSILDIQPK (MK15) induced the largest responses in terms of both breadth and magnitude, and were statistically associated with the alleles HLA-A*02:07, HLA-B*46:01, and HLA-C*01:02, which were under linkage disequilibrium (LD) association [21]. Presuming that the optimal epitope resides in the overlapping amino acid sequence between NI15 and MK15, that is, p24276–285
MYSPVSILDI (M10), we conducted a 51Cr release assay as previously described [22].

Results
Prediction of best-defined epitopes by the DSM and the peptide binding motif model

We have evaluated the predictive power of our DSM by testing its ability to predict epitopes within 52 15-mer peptides spanning the epitopes for seven HLA alleles enlisted in the Los Alamos database as best-defined epitopes. Overall, DSM ranked 43/52 (82.7%) of the best-defined epitopes correctly within the top five candidates, within which 14 epitopes ranked as the 1st, 11 as the 2nd, 7 as the 3rd, 3 as the 4th, then 8 as the 5th (Table S1). This was comparable to the HLArestrictor, where 37/52 (71.2%, 43/52 vs 37/52, p = 0.24 by Fisher's exact test) best-defined epitopes scored within the threshold of binding affinity without having 4 or more other candidate epitopes: 20 as SB, 10 as WB and 7 as CB. Table 1 summarizes the performance on epitope prediction by each model and dual positives by both models, according to their sensitivity, specificity, PPV and NPV. The performance of the DSM is similar to that of HLArestrictor. Interestingly, by introducing both models, specificity increased with significance (p<0.001), and an additive effect was seen in the PPV. We believe this is the first study to report a structure-based epitope prediction model with comparable or greater predictive power than a peptide-binding motif based model.

32/52 (61.5%) epitopes were detected as a significant epitope candidate by both models, 11/52 (21.2%) epitopes were detected only by the DSM, while 5/52 (9.6%) were detected only by HLArestrictor. 4/52 (7.7%) epitopes were not detected by either methods. Within the 14 epitopes not correctly predicted by HLArestrictor, incorrect epitopes were predicted in 7 epitopes. It is noteworthy that two epitopes, Nef157–177 PLRPMTYK (PK8) restricted by HLA-A*11:01 and Nef175–177 TQGYFPDWQNY (TY11) restricted by HLA-B*15:01 were detected as a NB by HLArestrictor, whereas they were ranked as the 2nd in PK8 and the 1st in TY11 in the DSM. Integrase179–188 AVFIHNFKRK (AK10) restricted by HLA-A*11:01 was predicted as a SB, but because there were 5 other SB candidates, 3 WB candidates and 1 CB candidate, this prediction was regarded as failure.

A striking feature of the DSM was that it had a high detection rate of best-defined epitopes independent of the peptide's length. The prediction rate of shorter epitopes (8 and 9-mer) was 27/31 (87.1%) while the rate for longer epitopes (10 and 11-mer) was 16/21 (76.2%), between which we found no significant difference by Fisher's exact test (p = 0.46). In contrast, the ability of HLArestrictor to accurately predict best-defined epitopes was highly dependent on epitope length, as the prediction rate of longer

Figure 1. Example of epitope prediction using the novel in silico docking simulation model. U_dock scores of the N-terminal (Row N1–N8) and C-terminal (Column C1–C8) was calculated and their sum was scored as the U_dock score (kcal/mol) of each 8 to 11-mer peptide’s. The lower score indicated stronger binding between the peptide and HLA. In this example, Gag p24258–272 KRVIILGLNK (KK10), well-known as one of the best-defined epitopes, scored −137.11 kcal/mol against HLA-B*27:05 and was the lowest (ranked as the 1st) among 26 variants in 15-mer peptide of Gag p24258–272 VGEIYKRVIILGLNK.

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<table>
<thead>
<tr>
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<th>HLArestrictor</th>
<th>Dual positives</th>
<th>p (mn Fisher's exact test)</th>
</tr>
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<td>Sensitivity</td>
<td>0.83</td>
<td>0.71</td>
<td>0.62</td>
<td>0.056</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.83</td>
<td>0.94</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPV</td>
<td>0.17</td>
<td>0.31</td>
<td>0.43</td>
<td>0.095</td>
</tr>
<tr>
<td>NPV</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 1. Evaluation of best-defined epitope prediction among docking simulation model, HLArestrictor, and positives in dual models.

Evaluation of best-defined epitope prediction among each model and positives in dual models were statistically evaluated, according to their sensitivity, specificity, positive prediction value (PPV) and negative prediction value (NPV) by maximum Fisher’s exact test. DSM: Docking simulation model.

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epitopes (11/21, 52.3%) was significantly lower than that of shorter ones (26/31, 83.9%) (p = 0.027).

Successful prediction with the DSM was dependent on the HLA allele and its peptides: in HLA-B*15:01, HLA-B*27:05 and HLA-B*35:01, all of the best-defined epitopes were ranked within the top 5. However, four best-defined epitopes restricted by HLA-B*57:01 and HLA-A*02:01 scored within the worst 5 candidates: Nef120-128 YFDPWQNYT, p15133-142 FLGKPSYK, R133-41 ALVEICTEM, and p24161-172 KAFSPVIMP.

Optimal epitope prediction to analyze HIV-1 CRF01_AE Gag ELISPOT assay data using two in silico models

We next applied the model to predict optimal epitopes against HIV-1 CRF01_AE Gag based on our previously obtained results in a Thai HIV cohort study [21]. In total, 31 peptide-HLA associations were analyzed: 5 in HLA-A, 13 in HLA-B, and 13 in HLA-C (Table S2). Among these, 10 overlapping peptides spanned previously reported epitopes (6 were best-defined epitopes and 4 were published but not enlisted as best-defined epitopes). In the DSM, 9/10 (90%) reported epitopes were successfully ranked within the 5th as significant candidate epitopes, and all of the six best-defined epitopes ranked either the 1st or 2nd. In HLA-A restrictor, 8/10 (80%) epitopes were predicted as significant binders; 3 as SB, 4 as WB, and 1 as CB, but 2 epitopes (best-defined epitopes HLA-A*02:07-restricted YI9, and HLA-B*46:01-restricted YI9) were not predicted as significant binders. HLA-A restrictor also predicted another 16 sequences as potential epitope candidates: 1 as SB, 12 as WB, and 2 as CB. Intriguingly only one WB candidate was ranked within the top five by the DSM, reflecting a considerable degree of discrepancy between the two prediction methods.

6 previously unreported peptides were predicted by both models: HLA-B*38:02-restricted p24307-320 MQMLKETI (rank 1st in DSM and WB in HLA restrictor), HLA-B*40:01-restricted p24311-321 QEVKNWMTI (2nd and SB), HLA-B*46:01-restricted p24277-283 RMYSPPVSIL (5th and SB), HLA-B*58:01-restricted p1779-86 YNTVVTILW (1st and WB), HLA-B*58:01-restricted p1777-86 SLNTVVTILW (4th and WB), HLA-C*01:02-restricted p24277-283 YSPVSDIL (2nd and WB in p24271-285 and 3rd and WB in p24276-290), HLA-C*01:02-restricted p24276-285 MYSPVSILDI (4th and WB both in p24271-285 and p24276-290), and HLA-C*01:02-restricted p24296-304 YVDFRYKTL (1st and WB).

Application of the in silico DSM to define the restricting HLA molecule

We conducted a 51Cr release assay with a truncated peptide titration spanning the overlapping region between Gag p24271-285 NKIVRMYSPPSILDI (NI15) and p24276-290 MYSPVSILDIRQGPK (MK15). These induced the largest responses both in breadth and magnitude in our previous study, and were statistically associated with HLA-A*02:07, HLA-B*46:01, and HLA-C*01:02, which we calculated to be under LD association [21]. We found strong killing against HLA-B*46:01 and HLA-C*01:02, with YI9 ranking slightly higher in YI9 encoding compatible or similar hydrophobic amino acids with the binding motif x[Y]xxxxxxx[I] in MI10 and xx[P]xxxxxx[J] in YI9. Together, these results indicate that the optimal epitopes MI10 and YI9 are equally likely candidates recognized by HLA-C*01:02, with YI9 ranking slightly higher in the DSM.

Discussion

In this study, we demonstrated that the structure-based DSM can predict the peptide binding affinity with various HLA molecules, independently of peptide binding motif information. To our knowledge, this novel DSM is the first model of its kind that succeeded in predicting HIV-1 CTL best-defined epitopes, with better or at least equivalent accuracy to the latest binding motif-based program. We also found a high detection rate of best-defined epitopes independent of peptide size in the DSM, while the detection rate significantly decreased with longer epitopes in the other model.

Historically, comparisons of epitope prediction methods has generally shown that peptide-binding motif based methods outperform structure-based methods [23]. However, the increased availability of crystal structures of MHC-peptide complexes is enabling the development of prediction methods using such structural models and the calculation of free energy of binding [23,24]. In the review by Liao et al [23], their comprehensive comparison of structure-based models and peptide-binding motif models in epitope prediction showed that the structure-based model was able to outperform all other methods except the ANN model, which performed equally well. In our novel program, we use a measure of the binding affinity between the HLA molecule and the peptides at four residues spanning the N- and C-terminal.

Table 2. Prediction of the HLA restriction of Gag p24276-285 MYSPVSILDI (MI10) and p24277-285 YSPVSDILDI (YI9) using in silico methods.

<table>
<thead>
<tr>
<th>HLA</th>
<th>Binding motif</th>
<th>Peptide</th>
<th>NI15</th>
<th>MK15</th>
<th>HLA restrictor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*02:07</td>
<td>x[L][D]xxxxxx[L]</td>
<td>MI10</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>YI9</td>
<td></td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>B*46:01</td>
<td>x[M][I]xxxxxx[YF]</td>
<td>MI10</td>
<td>15</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>YI9</td>
<td></td>
<td>19</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>C*01:02</td>
<td>x[AL]Pxxxxxx[L]</td>
<td>MI10</td>
<td>3</td>
<td>4</td>
<td>WB</td>
</tr>
<tr>
<td></td>
<td>YI9</td>
<td></td>
<td>2</td>
<td>3</td>
<td>SB</td>
</tr>
</tbody>
</table>

HLA restriction prediction against two reactive Gag peptides, Gag p24276-285 MYSPVSILDI (MI10) and p24277-285 YSPVSDILDI (YI9) was performed by the docking simulation model, and the binding motif HLA restrictor 1.2. The U_dock rank by the docking simulation model against MI10 and YI9 was analyzed in the original 15-mer peptides of Gag p24271-285 NKIVRMYSPPSILDI (NI15) and p24276-290 MYSPVSILDIRQGPK (MK15). SB: Strong Binder, WB: Weak Binder.
This covers not only the anchor position sites but also their flanking sites, which have a considerable effect on peptide-HLA binding; this may also have led to the high detection rate of best-defined epitopes independent of epitope size. Together with precise HLA crystal structure information, we have also incorporated a fine calculation model for binding affinity [18], giving the DSM a high detection rate of best-defined epitopes equivalent to that of the latest binding motif-based program.

Intriguingly there was a considerable degree of discrepancy between the two methods: 21.2% of the 52 best-defined epitopes were detected as significant epitope candidates only by the DSM, while 9.6% was detected only by the HLArestrictor. Furthermore, two epitopes which ranked within the bottom five by DSM were successfully predicted as a single candidate by HLArestrictor, whereas five epitopes which were not detected by HLArestrictor, were successfully predicted as the best candidates by the DSM. This result highlights the importance of combining programs with different approaches, for example those based on peptide binding motif information and those that do not require peptide binding motif information, consistent with previous report in class II HLA peptide binding prediction model [25].

We therefore applied both models to predict optimal epitopes in HIV-1 CRF01_AE Gag and found 8 previously unreported optimal epitopes supported by both models. These potential epitopes need to be further confirmed in vitro that they are true epitopes capable of stimulating T cell responses with either a 51Cr release assay or tetramer assay. However, since the DSM alone predicted 11 other candidates that were not predicted by the HLArestrictor, combining both models would be important to reduce the cost of such experiments. Furthermore a substantial number of OLPs were recognized using an ELISpot assay but within the peptides that induced a response, no epitope was predicted by the HLArestrictor. This DSM would save the cost of experiments by reducing 26 potential candidate peptides to five.

The ability of the DSM model to accurately predict peptides was dependent on the HLA molecule in question, and our results suggest that this is due to variations in the C-terminal binding groove. Four best-defined epitopes restricted by the alleles HLA-A*02:01 and HLA-B*57:01 ranked among the worst from the 22nd up to the 26th in our program. In HLA-A*02:01, both FK10 and AM9 coded Leucine (L) at the 2nd position of sequence, compatible with the HLA-A*02:01 binding motif at the B pocket and scored a low and therefore strongly binding U_dock score at 48.5 kcal/mol in AM9 (2nd) and YT9 

Intriguingly there was a considerable degree of discrepancy between alleles with/without original crystal structure information, further calculations will be warranted for the quality evaluation of DSM. Second, this is a computational epitope prediction model whose algorithm is solely based on the binding between the peptide and the HLA molecule. Although peptide-HLA binding is the most selective event for epitope determination [32], CTL activation is a multi-step process involving the processing of viral peptides by proteasome [22,33,34] and the recognition of the peptide-HLA complex by T cell receptors (TCRs) [35], both of which are not accounted for in the model.

In conclusion, we have shown here a novel in silico DSM which can be used for epitope mapping, and combined with a binding motif-based model, this will significantly reduce the required experimental burden for epitope identification in the development of a CTL-based vaccine for HIV.

### Supporting Information

**Figure S1** Identification of HLA-B*46:01/C*01:02-restricted Gag p24276-285 MI10 and p24277-285 Y19 by a 51Cr release assay. 51Cr release assays under HLA-B*46:01/ C*01:02-matched conditions were performed for each peptide. Significant % lysis was found in target cells pulsed with Gag p24276-285 MI10: MYSVPWSILDI and p24277-285 Y19: YSPVSILDI. (PPTX)

**Table S1** Predicted best-defined epitopes using the docking simulation model and a comparison with HLArestrictor. The docking simulation model was applied to predict epitopes within 15-mer peptides spanning best-defined epitopes and compared with those predicted with the HLArestrictor. The U_dock score and their rank were calculated for each peptide in the docking simulation model, while with HLArestrictor the affinity thresholds of SB: Strong Binder, WB: Weak Binder, and CB: Combined Binder, and Non-binder were given, according to their definitions. (XLS)
underlined sequences indicate positive candidates in dual models. Bold, performed by our novel docking simulation model and HLArestrictor. Reported HIV-1 CRF01_AE Gag epitope candidates detected by Strong Binder, Weak Binder, and Combined Binder.

**References**


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**Author Contributions**

Conceived and designed the experiments: TM MY KA. Performed the experiments: MM KM TM MT BS. Analyzed the data: MM KM TM. Contributed reagents/materials/analysis tools: TM MY KA. Wrote the paper: MM KA.