Electronic Supplementary Material (ESI) for Analyst.

Rapid quantification of the malaria biomarker hemozoin by improved biocatalytically initiated precipitation atom transfer radical polymerizations

Samuel Raccio[1], Jonas Pollard[1], Ashley Djuhadi[1], Sandor Balog[1], Michela M. Pellizzoni[1], Kyle J. Rodriguez[1], Omar Rifaie-Graham[#1], Nico Bruns[1,2]*

*Corresponding author: nico.bruns@strath.ac.uk

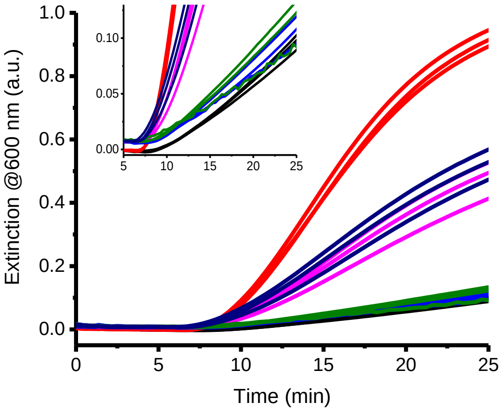
[1]Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, 1700 Fribourg, Switzerland

[2]Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, United Kingdom

[#]Current address: Department of Materials and Department of Bioengineering, Institute of Biomedical Engineering, Imperial College London, Exhibition Road, London SW7 2AZ, United Kingdom

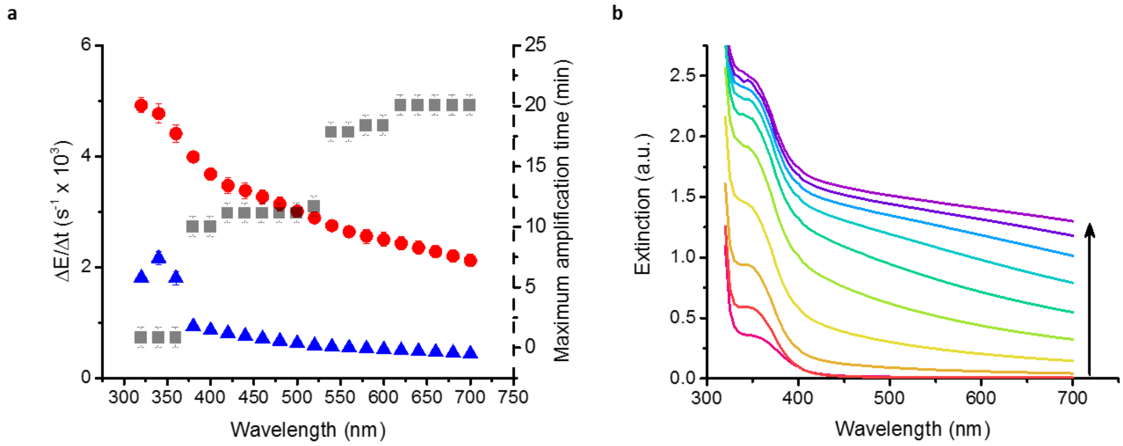
Supplementary Information

1. Supplementary data and information:



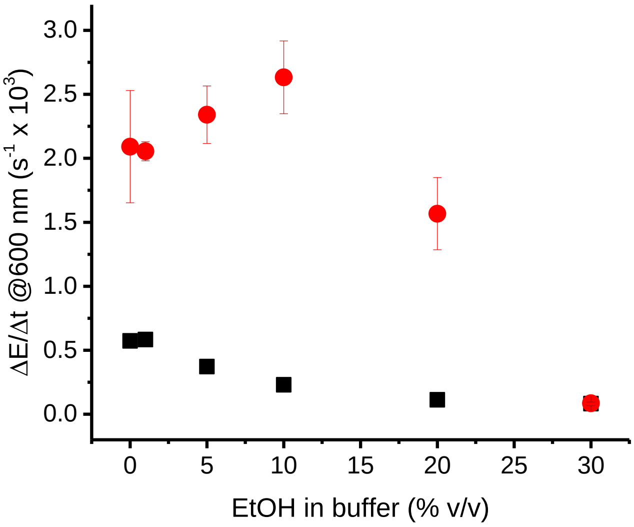
Supplementary Figure 1. Influence of crosslinker on the precipitation polymerization. 100 ng mL^{-1} sHz catalyzed (red) and non-catalyzed (black) reactions in the absence of bisacrylamide (BA) were compared to catalyzed and non-catalyzed reaction in the presence BA. NIPAAm:BA 99:1 catalyzed (pink) and non-catalyzed (blue). NIPAAm:BA 90:10 catalyzed (navy blue) and non-catalyzed (green). Reaction conditions were 800 mM monomer, 36 mM HEBIB, 80 mM Asc, 100 mM Pyr, 170 µM SDS, 37 °C and pH 6.

Crosslinkers such as bisacrylamide (BA) are often used in the synthesis of PNIPAAm microgel particles.[62, 63] In order to test whether crosslinkers could make the precipitation polymerization assay more efficient, a fraction of the monomer was replaced by BA at ratios NIPAAm:BA of 99:1 and 90:10 in reactions catalyzed by 100 ng mL^{-1} sHz (**Supplementary Figure 1**). The addition of BA lowered the rate of turbidity formation. The non-catalyzed reactions were not significantly affected by the BA. Most likely, the addition of crosslinker led to more compact PNIPAAm particles that scattered less light. As BA decreased the differentiation between catalyzed and non-catalyzed reactions, its use is not beneficial to the hemozoin assay.



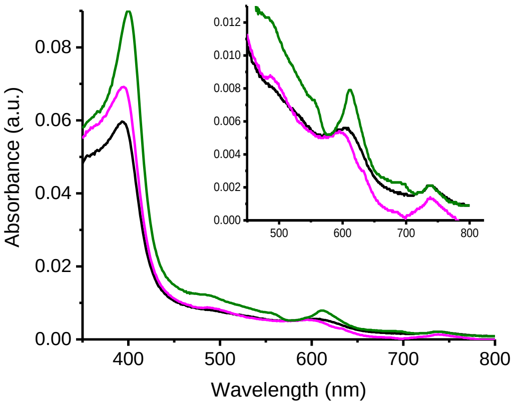
Supplementary Figure 2. Influence of the measuring wavelength on the rate of turbidity formation of a precipitation polymerization catalyzed by 100 ng mL^{-1} sHz (red dot) and a non-catalyzed reaction (blue triangle) (average of n = 3 and SD). The amplification time of the non-catalyzed reaction is displayed on the second y axis (grey square, average of n = 3 and SD). **b** Stack of UV-vis spectra for a single reaction catalyzed by 100 ng mL^{-1} sHz recorded every 90 s over 15 min. For **a** and **b**, reaction conditions were 800 mM NIPAAm, 36 mM HEBIB, 80 mM Asc, 170 μM SDS, and pH 7.

For the hemozoin assay, turbidity is measured by passing a beam of light through the sample and recording extinction in a UV-vis spectrometer. When nanoparticles form in suspension, light is scattered in all directions resulting in a decrease in the light intensity measured at the exit of the sample. The intensity of the scattered light increases with decreasing wavelength, i.e. near-ultraviolet light scatters more intensely than visible and near-infrared light. The impact of the measuring wavelength was studied by measuring samples containing 100 ng mL^{-1} hemozoin and samples without hemozoin. The reactions were followed by spectral scans between 320 to 700 nm. Shorter wavelengths resulted in higher rates of turbidity formation and shorter maximum amplification times. For the catalyzed reaction, $\Delta E/\Delta t$ was 2.12 ± 0.11 ms^{-1} at 700 nm and 4.92 ± 0.13 ms^{-1} at 320 nm. The non-catalyzed reaction resulted in $\Delta E/\Delta t$ = 0.44 ± 0.01 ms^{-1} at 700 nm and 1.81 ± 0.01 ms^{-1} at 320 nm. The maximum amplification time reduced from 20 ± 0.5 min to 1 ± 0.5 min by measuring at 700 nm or 320 nm. (Please note: The abrupt changes in amplification time at 550 nm and 375 nm are caused by the automatic data analysis script due to the low time resolution of these measurements.) Sodium pyruvate absorbs strongly below 400 nm (**Supplementary Figure 2 a**). Moreover, the ascorbate degradation product L-threosone also absorbs below 400 nm and contributes to the measured extinction (**Supplementary Figure 2 b**).[69] In order to measure at a short wavelength while avoiding a too high absorption of pyruvate and L-threosone, a wavelength of 410 nm was used for further data acquisition unless otherwise noted.

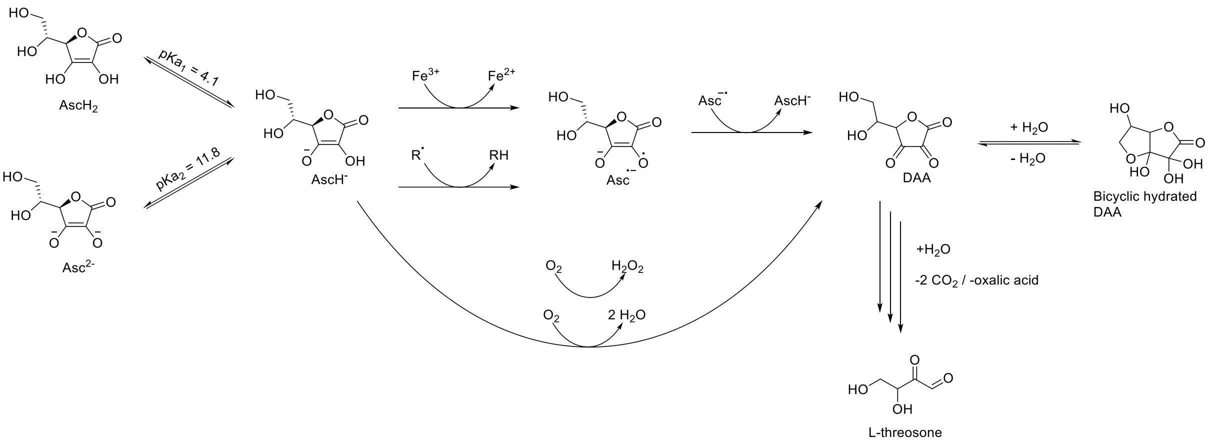


Supplementary Figure 3. Influence of the concentration of the cosolvent ethanol on the non-catalyzed reactions (black square) and on precipitation polymerizations catalyzed by 100 ng mL^{-1} sHz (red dot) (average of n = 3 and SD). Reaction conditions were 800 mM NIPAAm, 100 mM HEBIB, 80 mM NaAsc, 37 °C and pH 6.

Reactions catalyzed by 100 ng mL^{-1} sHz were not significantly affected by the addition of ethanol up to 10 % v/v (**Supplementary Figure 3**). At 20 % the turbidity level decreased and at 30 % no precipitation was observed. With a high amount of EtOH as co-solvent, the chains remain soluble instead of precipitating. For the non-catalyzed reactions, the rate of turbidity formation decreased with ethanol concentration, possibly because ethanol inhibits the formation of hydroxyl radicals that may form during the decomposition of ascorbate.[47, 49] The optimum ethanol concentration for the assay was found to be 10 vol % because it resulted in the highest resolution between catalyzed and non-catalyzed reactions.

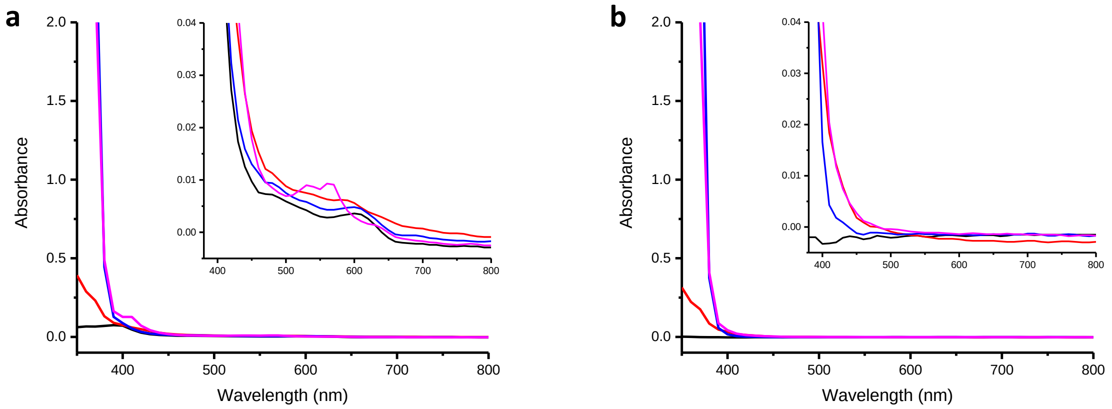


Supplementary Figure 4. Influence of concentration of sodium dodecyl sulfate on the UV-vis spectra of hemozoin (1000 ng mL^{-1}). 0.170 mM SDS (pink), 34.7 mM SDS (green), and without SDS (black).

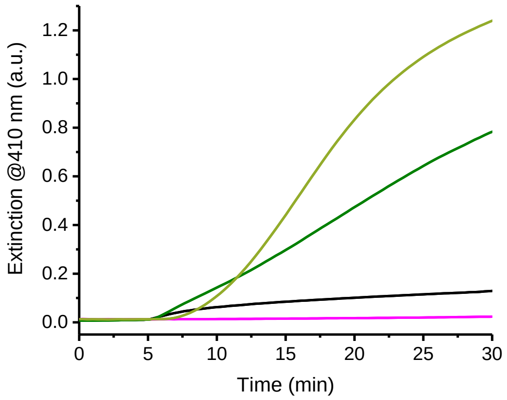


Supplementary Figure 5. Ascorbate degradation pathway at neutral pH in the presence of oxygen.

Ascorbate is known to react with radicals, metal traces, and oxygen which leads to the generation of a wide variety of compounds coming from a complex degradation pathway (**Supplementary Figure 5**).[1-4] The protonation of ascorbate varies as a function of pH. Below pH 4.1, the main ascorbate species present in solution is fully protonated ($AscH_2$). Between pH 4.1 and 11.8, ascorbate is mostly present in the form of the monoanion ($AscH^-$) and at higher pH values, the dianion (Asc^{2-}) is dominant. At pH 7.4, 99.95% of ascorbate is in the form of the monoanion, $AscH^-$. When in contact with free radicals ($R^{\bullet}$) or metal traces (Fe(III) or Cu(II)), $AscH^-$ forms the monodehydroascorbate radical anion ($Asc^{\bullet -}$). At lower concentrations, $Asc^{\bullet -}$ can remain dominant due to the stabilization of the radical by delocalization over the π-system and the tricarbonyl moiety. At high concentrations, $Asc^{\bullet -}$ reacts by dismutation, and $AscH^-$ and dehydro-L-ascorbic acid (DAA) are formed.[5, 6] $Asc^{\bullet -}$ dismutation is a very fast process with $K_{obs} = 1.4 \times 10^5\ M^{-1}\ s^{-1}$ at pH 7.4. In phosphate buffer, this rate constant increases by an order of magnitude. Further, degradation of DAA into L-threosone as a main component has been shown by *Nishikawa et al.*.[7] Concerning the reaction between oxygen and ascorbate, two possible reaction pathways are debated leading to the formation of either hydrogen peroxide or water.[8-10] This oxidation in absence of catalyst has been referred to sodium ascorbate true oxidation by *Buettner et al.*.[3] They have shown that the autoxidation of ascorbate is pH dependent and very slow for $AscH_2$. The ascorbate oxidation by oxygen can be highly promoted by the presence of metal traces such as iron or copper at neutral and basic pH.[11] Depending on the metal traces present in the buffer salt, ascorbate decomposes by 10 to 30 % within 15 min. When metal traces are removed, the decomposition is reduced to 0.05 % after 15 min. The use of ion exchange columns or chelating agents, such as diethylenetriaminepentaacetic acid (DETAPAC) or deferoxamine mesylate, can be used to sequester metal traces and thus prevent ascorbate decomposition.[12] Iron traces can also catalyze the Haber-Weiss chain reaction leading to the formation of the hydroxyl radical which is reactive enough to initiate polymerization.[13] Ascorbate chemistry is difficult to predict since it can undergo many possible reaction mechanisms. Understanding its behavior in a given system requires experimental controls to understand which pathway and decomposition products are being formed.



Supplementary Figure 6. *UV-Vis spectra of **(a)** 1000 ng mL^{-1} hemozoin and **(b)** without hemozoin after 3 min incubation at 45 °C under different conditions: sodium phosphate buffer (pH 7.0, 0.1 M, 10 % v/v EtOH) (black), buffer with 80 mM ascorbate (red), buffer with 650 mM pyruvate (blue), and buffer with 80 mM ascorbate and 650 mM pyruvate (pink). UV-vis measurements were taken at a scan rate of 50 nm s^{-1} with a resolution of 10 nm.*



Supplementary Figure 7. Control reactions used to determine the effect of pyruvate on the non-catalyzed precipitation polymerization. Non-catalyzed reaction with 650 mM pyruvate (pale green), non-catalyzed reaction in the presence of 650 mM pyruvate without HEBIB (pink), and non-catalyzed reaction without HEBIB and without pyruvate (black). For comparison, the non-catalyzed reaction is also shown (dark green). Reaction conditions: 800 mM NIPAAm, 36 mM HEBIB, 80 mM Asc, 45 °C, pH 7.

2. MATLAB Script

```
%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%96-Wellplate kinetics analyzer
%Jerome Brouchoud, AMI Master Student, 31.03.2017
%V8
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%TO DO
%   commenter slope determination
%   améliorer importation well plate
%   export format .mat ne fonctionne pas (garder seulement CSV)
%   grossir le trait export global
%   recoder la selection des outliers en enlevant une boucle
%   diminuer le nombre de tangente affichée
%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
clc
clear all
close all
empl=cd;
%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%PARAMETERS
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
dim=7; %number of point of the linear regression
v=1;%visibility of slope determination
abs=['1' '2' '3' '4' '5' '6' '7' '8' '9' {'10'} {'11'} {'12'}];
ord=['A' 'B' 'C' 'D' 'E' 'F' 'G' 'H'];
%res.value=[0 0.1 1 10 100 200 400 800];
for i=1:12
    for j=1:8
        WellId(i,j)=strcat(ord(j),abs(i));
    end
end
WellId=reshape(WellId,96,1);



%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%DATA ACQUISITION
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%WELL PLATE FILE
%Selection and opening
disp('Choose file from plate reader')
[pdataname,pdatapath]=uigetfile('.col','Choose file from plate reader');
pdatafile=strcat(pdatapath,pdataname);
cd(pdatapath)
pfile=fopen(pdatafile,'r'); %opening of the file
disp(pdatafile)
name = pdataname(1:end-4);%remove extension ".col"
name = strrep(name,'.',',')

%Reading data header
MainHeader=fgetl(pfile);%text 1st line
TimeSteps=fscanf(pfile,'%f',1);%number of time steps
PlatDim=fscanf(pfile,'%f',2) ;%dimension of the well plate
NumberOfWells=PlatDim(1)*PlatDim(2); %number of wells

%Reading time information
for n=1:TimeSteps %acquisition of the time HH:MM:SS
    HH=fscanf(pfile,'%f',[1]);%HH
    fscanf(pfile,'%c',[1]);%:
    MM=fscanf(pfile,'%f',[1]);%MM
    fscanf(pfile,'%c',[1]);%:
```

```
    SS=fscanf(pfile,'%f',[1]);%SS
    time(n)=3600*HH+60*MM+SS; %conversion in seconds
end
time=time-time(1); %put t1 at 0...


%WELL PLATE ATTRIBUTION FILE
control=0;
if(exist('WellsLabel.mat','file'))
    load('WellsLabel.mat');
    disp(WellsLabel)
    control=input('Is the wells attribution is correct? Yes=1, No=0 \n');
end

if(~control)
    %selection of the file
    disp('Choose file containing wells information')
    [pwellname,pwellpath]=uigetfile('.xlsx','Choose file containing wells
information');
    pwellfile=strcat(pwellpath,pwellname);
    disp(pwellfile)

    %Reading wells information
    [~,~,WellsLabel1]=xlsread(pwellfile,'b2:m9')

    for i=1:8
        for j=1:12
            WellsLabel1{i,j}=string(WellsLabel1{i,j});
        end
    end

    [~,~,l]=xlsread(pwellfile,'b1:m1')
    [~,~,l2]=xlsread(pwellfile,'a2:a9')
    WellsLabel=cell2table(WellsLabel1);


   WellsLabel.Properties.VariableNames=l;
   WellsLabel.Properties.RowNames=l2;



    %Saving
    save('WellsLabel.mat','WellsLabel');

end
ExperimentLabel=reshape(table2array(WellsLabel)',NumberOfWells,1); %make a colum
with WellsLabel



%READING DATA PRO WELL
Data=[];
Comments=cell(1,NumberOfWells);
Validity=[];
Slope=zeros(1,NumberOfWells);

for i=1:NumberOfWells
    overrange=0;

     for j=1:TimeSteps
            x=fscanf(pfile,'%f',[1]);
            if ~size(x,1) %if data is not a number X.XXX_ pass 5 chars
                fscanf(pfile,'%c',[5]);
                x=cr(j-1);%attribue la valeur précédente
                overrange=1;

            end
            cr(j)=x;
        end


    if(strcmp(ExperimentLabel(i),'x')) %check if data is 'x'
        Validity(i)=-1;%put the validtiy at -1
        Data(i,:)=zeros(1,TimeSteps);

       % fseek(pfile,8*TimeSteps,0);
        %fgetl(pfile);%read and dont save the line
        %pos=ftell(pfile);
    else
        Validity(i)=1;%put the validity at 1

        Data(i,:)=cr;

    end

    if overrange
        Comments(i)=java.lang.String('overrange');
    else
        Comments(i)=java.lang.String('');
    end

    %system('PAUSE')
  %  Data(i,:)
       % system('PAUSE');
end

fclose(pfile);

%PREPARATION TABLE OF DATA
id=(1:NumberOfWells)' ;
dtt=sortrows(table(ExperimentLabel,WellId,Data,Validity',Comments',Slope'),1);
dtt.Properties.VariableNames = {'Label' 'WellId' 'Data' 'Validity'
'Comments','Slope'};
dtt=table(id,dtt.Label,dtt.WellId  ,dtt.Data,dtt.Validity,dtt.Comments,dtt.Slope);
dtt.Properties.VariableNames = {'Id' 'Label' 'WellId' 'Data' 'Validity'
'Comments','Slope'};
disp(dtt);
WP(dtt.WellId,dtt.Validity,dtt.Label,PlatDim,name)
%%
if(1) % outlier and slope calculation

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%SELECTION OF OUTLIER
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
FamName=unique(dtt.Label);
mkdir('Outlier') % create a folder
cd('Outlier') % go in the folder
j=1;

for i=1:length(FamName) % loop on the family
  %Plotting
    if dtt.Validity(j)<0 % do not plot non valid data
       j=j+1 ;
       continue
    end
    k=1;
    lgd=[];


    fig=figure('units','normalized','outerposition',[0 0 1 1]);
    hold on

    while strcmp(dtt.Label(j),FamName(i))
       p(k)=plot(time,dtt.Data(j,:));
       lgd=[lgd;strcat(num2str(dtt.Id(j),'%03i'),'-',dtt.WellId(j))];
       k=k+1 ;
       j=j+1;
    end

    fig.Name=char(strcat(string(name),'-',string(cell2mat(FamName(i)))));
    title(fig.Name);
    xlabel('Time [s]');
    ylabel('Extinction [-]');
    legend(lgd);
    for l=1:k-1
        p(l).Color=rbcol(l,k-1);
        p(l).LineWidth=1;
    end

   %Selection of outlier

    while 1 % 0 -> OK
       dtr=input('Indicate the data to remove (0 if none)\n ');
       if(isempty(dtr))
           dtr=0;
end

       if ~dtr
            break
        end; %stop the loop if dtr=0
       if (dtr>j-1) || (dtr<j-k+1)
            continue
        end


        cp=dtr-j+k; %index of the plot
        nl=char(lgd(cp));%legend to modify

        if dtt.Validity(dtr) %plot -> dash, X in legend
            p(cp).LineStyle='--';
            nl(1)='X';
            comment=input('Add a comment \n','s');

dtt.Comments(dtr)=java.lang.String(strcat(dtt.Comments(dtr),'/',comment));
        else
            p(cp).LineStyle='-'; %plot -> continue, 0 in legend
            nl(1)='0';
        end
        dtt.Validity(dtr)=~dtt.Validity(dtr); %change the valitidy
        lgd(cp)=cellstr(nl);%update the legend
        legend(lgd);
    end

    %save and close
    hold off
    print(fig,fig.Name,'-dpng');
    saveas(fig,strcat(fig.Name,'.fig'));
    close all

end
cd ..
WP(dtt.WellId,dtt.Validity,dtt.Label,PlatDim,name)



%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%SLOPE DETERMINATION
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%


mkdir('Slope')
cd('Slope')
```

```
k=1;
for i=1:NumberOfWells
if(dtt.Validity(i)~=1)
    continue
end

    fig=figure('units','normalized','outerposition',[0 0 1 1]);
    if ~v
        set(fig,'visible','off');
    end
    fig.Name=char(strcat(string(name),'-',string(dtt.Label(i)),'-
',string(dtt.Id(i))));
    title(fig.Name);
    subplot(2,2,[1 3]);

    y=dtt.Data(i,:);
    pt=size(y,2)-1;

    x=time;
    x=[ones(length(x),1) x'];


subplot(2,2,[1 3]);





hold on
lgd=['K'];

plot(x(:,2)',y,'LineWidth',2,'color','k');
xlabel('Time [s]');
ylabel('Extinction [-]');
ax=gca;
ax.YLim = [0.9*min(y) max(y)*1.1];
title('Fit-Slope');
l=1;
for j=1:pt-dim

   xr=x(j:j+dim,:);
   yr=y(j:j+dim);

   P(j,:) = xr\yr';


   ycalc=P(j,:)*x';
   yrcalc=ycalc(j:j+dim);



   Rsq(j) = 1 - sum((yr - yrcalc).^2)/sum((yr - mean(yr)).^2);

   if (isnan(Rsq(j)) || Rsq(j)<0) %% solve issue division by 0
       Rsq(j)=0;
   end
   nbp=15;%number of line

   if(mod(j,nbp)==0)
   plot(x(:,2)',ycalc,'color',rbcol(l,(pt-dim)/nbp));
   lgd=[lgd,strcat(string(P(j,2)),' / ',string(Rsq(j)))];
   lgdd=legend(lgd,'location', 'southeast'  );
   l=l+1;
%pause(0.2)
   end

end
title(lgdd,'Slope / R^2');
```

```
hold off
%close all
%pause(2)


subplot(2,2,2);

hold on
yyaxis left
%plot(1:pt-dim,p,1:pt-dim,mu)
%plot(1:pt-dim,P(:,2));
plot(x(1:pt-dim,2)',P(:,2));

ylabel('Slope [s^{-1}]');
yyaxis right;
plot(x(1:pt-dim,2)',Rsq);
ylabel('R^2');
xlabel('Time [s]');
title('Max slope / R^2');


hold off
%pause(2)

subplot(2,2,4);
plot(x(:,2)',y,'LineWidth',2,'color','k');
hold on

ax=gca;
lgd=['K',];
[MaxA,MaxI]=max(P(:,2).*Rsq');
ycalc=P(MaxI,:)*x';
plot(x(:,2)',ycalc,':');
plot(x(MaxI:MaxI+dim,2)',y(MaxI:MaxI+dim),'LineWidth',2,'color','r');
xlabel('Time [s]');
ylabel('Extinction [-]');
ax.YLim = [0.9*min(y) max(y)*1.1];
lgd=[lgd,strcat(string(P(MaxI,2)),' / ',string(Rsq(MaxI)))];
dtt.Slope(i)=P(MaxI,2);
%system('PAUSE')
title('Decision');
lgdd=legend(lgd,'location','southeast');
title(lgdd,'Slope / R^2');

   print(fig,fig.Name,'-dpng');
   saveas(fig,strcat(fig.Name,'.fig'));
  close all
end

end
%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%EXPORT GENERATION
%%%%%%%%%%%%%%%%%%%%%%%%%%%%
cd ..
mkdir('Table')
cd('Table')

[~,~,npc]=unique(dtt.Label) % number of slope
nos=max(histc(npc,unique(npc)) )

toDelete = dtt.Validity < 0;
dtt(toDelete,:) = [];


pfileG=fopen('global.csv','w');
%pfileP=fopen('selection.csv','w');
fprintf(pfileG,'Time,');
```

```
for i=1:size(dtt,1)
    d=string(dtt.Label{i});
    fprintf(pfileG,'%s,',d);
end
fprintf(pfileG,'\n');

fprintf(pfileG,'s,');
for i=1:size(dtt,1)
    fprintf(pfileG,'-,');
end
fprintf(pfileG,'\n');
fprintf(pfileG,',');

for i=1:size(dtt,1)
    d=string(dtt.Comments{i});
    fprintf(pfileG,'%s,',d);
end
fprintf(pfileG,'\n');

fprintf(pfileG,',');

for i=1:size(dtt,1)

    fprintf(pfileG,'%f,',dtt.Validity(i));
end
fprintf(pfileG,'\n');


for i=1:TimeSteps
    fprintf(pfileG,'%f,',time(i));
    for j=1:size(dtt,1);
    d=dtt.Data(j,i);

    fprintf(pfileG,'%f,',d);
    end
    fprintf(pfileG,'\n');
end

fclose(pfileG)

toDelete = dtt.Validity < 1;
dtt(toDelete,:) = [];


pfileG=fopen('selection.csv','w');
%pfileP=fopen('selection.csv','w');
fprintf(pfileG,'Time,');
for i=1:size(dtt,1);
    d=string(dtt.Label{i});
    fprintf(pfileG,'%s,',d);
end
fprintf(pfileG,'\n');

fprintf(pfileG,'s,');
for i=1:size(dtt,1)
    fprintf(pfileG,'-,');
end
fprintf(pfileG,'\n');
fprintf(pfileG,',');

for i=1:size(dtt,1)
    d=string(dtt.Comments{i});
    fprintf(pfileG,'%s,',d);
end
fprintf(pfileG,'\n');

fprintf(pfileG,',');
```

```
for i=1:size(dtt,1)

    fprintf(pfileG,'%f,',dtt.Validity(i));
end
fprintf(pfileG,'\n');


for i=1:TimeSteps
    fprintf(pfileG,'%f,',time(i));
    for j=1:size(dtt,1);
    d=dtt.Data(j,i);

    fprintf(pfileG,'%f,',d);
    end
    fprintf(pfileG,'\n');
end

fclose(pfileG)


cd ..
mkdir Result
cd Result



 FamName=unique(dtt.Label);
 fig=figure('units','normalized','outerposition',[0 0 1 1]);
hold on
    fig.Name=char(strcat(string(name),' - all valide data'));
    title(fig.Name);
 lgd=[];

 for i=1:size(FamName,1)
      plot(0,0,'color',rbcol(i,size(FamName,1)));
 end


  for i=1:size(FamName,1)
      sel=strcmp(FamName(i),dtt.Label);
      dtt2=dtt(sel,:);
      res.label(i)=FamName(i);
      res.mean(i)=mean(dtt2.Slope);
      res.std(i)=std(dtt2.Slope);
      res.max(i)=max(dtt2.Slope);
      res.min(i)=min(dtt2.Slope);
      res.RSD(i)=res.std(i)/res.mean(i);
      %SSlope(i,:)=dtt2.Slope;
      res.number(i)=length(dtt2.Slope);
      res.value(i)=i;
      sto=nan(1,nos);
      sto(1:length(dtt2.Slope))=dtt2.Slope'
      res.slope(i,:)=sto

    % system('PAUSE');
          plot(time,dtt2.Data,'color',rbcol(i,size(FamName,1)));
         % p(i,:).Color=rbcol(i,size(FamName,1));

          lgd=[lgd,FamName(i)];
         %system('PAUSE');



  end
       legend(lgd);
       xlabel('time [s]');
       ylabel('extinction [-]');
       print(fig,fig.Name,'-dpng');
```

```
        saveas(fig,strcat(fig.Name,'.fig'));



res=table(res.value',res.label',res.mean',res.std',res.RSD',res.max',res.min',res.n
umber',res.slope);

       res.Properties.VariableNames={'Value' 'Label' 'Mean' 'STD' 'RSD' 'MAX' 'MIN'
'number' 'slope'};
  %      res.Properties.RowNames=res.Label;
writetable(res,'slope.csv') ; %pcr
save('res.mat','res');

catplot=isempty(str2num(char(res.Label)));
fig=figure('units','normalized','outerposition',[0 0 1 1]);
hold on

if(catplot)
xticks(res.Value);
xticklabels(res.Label);
fig.Name=char(strcat(string(name),' -Slope VS Category'));
xlabel('Category');
aax=axis;
aax(1)=0.5;
aax(2)=length(res.Label)+0.5;
axis(aax);

else
res.Value=str2num(char(res.Label));
fig.Name=char(strcat(string(name),' -Slope VS Concentration'));
x=res.Value;
y=res.Mean;

[p,s] = polyfit(x,y,1);
[yfit,dy] = polyconf(p,x,s,'predopt','curve');
line(x,yfit,'color','r','LineWidth',2)
line(x,yfit-dy,'color','r','linestyle','--')
line(x,yfit+dy,'color','r','linestyle','--')
xlabel('sHz [ng/mL]');

end

ylabel('slope [s]');

title(fig.Name);
errorbar(res.Value,res.Mean,res.STD,'s');
axis('auto y');

%fplot(@(x) res.Mean(1)+res.STD(1))





%dr=x\y;

%define plot range
%xp=[ones(1,2);linspace(min(res.Value)*0.9,max(res.Value)*1.1,2)];
%yp=dr'*xp;

%plot(xp(2,:),yp);


hold off
res
print(fig,fig.Name,'-dpng');
 saveas(fig,strcat(fig.Name,'.fig'));
```

```
%close all;




cd(empl);
%
function [col] = rbcol(x,y)

x=1530/y*x;

if x<255
    col(1)=255;
    col(2)=x;
    col(3)=0;
elseif x<510
    col(1)=510-x;
    col(2)=255;
    col(3)=0;
elseif x<765
    col(1)=0;
    col(2)=255;
    col(3)=x-510;
elseif x<1020
    col(1)=0;
    col(2)=1020-x;
    col(3)=255;
elseif x<1275
    col(1)=x-1020;
    col(2)=0;
    col(3)=255;
else
    col(1)=255;
    col(2)=0;
    col(3)=1530-x;
end



col=col/255;
end

function WP(wellId,validity,label,PlatDim,name)
s=PlatDim(2);
PlatDim(2)=PlatDim(1);
PlatDim(1)=s;

 abs=['1' '2' '3' '4' '5' '6' '7' '8' '9' {'10'} {'11'} {'12'}];
 ord=['A' 'B' 'C' 'D' 'E' 'F' 'G' 'H'];

 for x=1:PlatDim(2)
     for y=1:PlatDim(1)
        % WPL(x,y)=strcat(ord(x),abs(y));
         [t,loc]=ismember(strcat(ord(x),abs(y)),wellId);
         if validity(loc)==-1
             coo='k';
         elseif validity(loc)==0
             coo='r';
         else
             coo='g';
         end
         WPC(x,y)=coo;
         WPL(x,y)=label(loc);
     end
 end

          fig=figure('units','normalized','outerposition',[0 0 1 1]);
 fig.Name=strcat(name,'-WellPlate');
 title(fig.Name);

  for x=1:PlatDim(1)
     for y=1:PlatDim(2)

         ll=char(WPL(PlatDim(2)+1-y,x));
         rectangle('position',[x-0.5,y-0.5,1,1]);
         rectangle('position',[x-0.5,y-0.5,1,1],'FaceColor',WPC(PlatDim(2)+1-
y,x),'Curvature',[1 1]);
         text(x-0.2,y,ll);
     end
  end


   for x=1:PlatDim(1)
     text(x,PlatDim(2)+0.75, abs(x),'FontSize',14);

 end

 for y=1:PlatDim(2)
     text(0.25,y, ord(PlatDim(2)+1-y),'FontSize',14);

 end

 axis off;
 print(fig,fig.Name,'-dpng');
  saveas(fig,strcat(fig.Name,'.fig'));



close all;
end
```

3. References

1. N. Smirnoff, *Free Rad. Biol. Med.*, 2018, **122**, 116-129.
2. N. Smirnoff, *Ann. Bot.*, 1996, **78**, 661-669.
3. G. R. Buettner and B. A. Jurkiewicz, *Radiat. Res.*, 1996, **145**, 532-541.
4. J. Du, J. J. Cullen and G. R. Buettner, *Biochim. Biophys. Acta*, 2012, **1826**, 443-457.
5. G. R. Buettner and B. A. Jurkiewicz, *Free Rad. Bio. Med.*, 1993, **14**, 49-55.
6. G. R. Buettner, *Arch. Biochem. Biophys.*, 1993, **300**, 535-543.
7. Y. Nishikawa, Y. Toyoshima and T. Kurata, *Biosci. Biotechnol. Biochem.*, 2001, **65**, 1707-1712.
8. A. Serpen and V. Gökmen, *Food Chem.*, 2007, **104**, 721-725.
9. R. J. Wilson, A. E. Beezer and J. C. Mitchell, *Thermochim. Acta*, 1995, **264**, 27-40.
10. I. Oey, P. Verlinde, M. Hendrickx and A. Van Loey, *Eur. Food Res. Technol.*, 2006, **223**, 71-77.
11. G. R. Buettner, *J. Biochem. Biophys. Methods*, 1988, **16**, 27-40.
12. G. R. Buettner and P. G. Czapski, *Free Radic. Res. Commun.*, 1986, **1**, 349-353.
13. F. Haber and J. Weiss, *Naturwissenschaften*, 1932, **20**, 948-950.